This book composes the abstracts of the presentations for the platform and poster sessions of the 6th World Congress/22nd Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), conducted at the Estrel Hotel in Berlin, Germany from 20-24 May 2012. The abstracts are reproduced as accepted by the scientific committee of the meeting and appear in order of abstract code, in alphabetical order per presentation type. The poster spotlight abstracts and poster corners abstracts are included in the list of poster abstracts.
In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists, and others interested in environmental issues such as managers and engineers. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in 1979 to fill the void. Based on growing membership, meetings attendance, and publications, the forum was needed.

A unique strength of SETAC is its commitment to balance the interests of academia, business, and government. The Society by-laws mandate equal representation from these three sectors for World Council Officers, Board of Directors / Council Members, and Committee members. And although there is no control mechanism, the proportion of members from each of the three sectors has remained nearly equal over the past 30 years.

Like many other professional societies, SETAC publishes esteemed scientific journals and convenes annual meetings replete with state-of-the-science poster and platform presentations. Because of its multidisciplinary approach, however, the scope of the science of SETAC is much broader in concept and application than that of most other societies.

SETAC is concerned about global environmental issues. Its members are committed to good science worldwide, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur.

SETAC was founded in North America but membership was open to environmental scientists worldwide. SETAC Europe was organized in 1989; SETAC Asia / Pacific in 1997 and SETAC Latin America in 1999. Members voted overwhelmingly in 2001 to combine these “geographic units” into one global society to form the SETAC World Council. SETAC meets the professional needs of individuals at local and regional levels throughout all geographic units, throughout national branches and chapters (Argentina, Brazil, United Kingdom, Central and Eastern Europe, Africa and soon-to-be organized Japan), through regional chapters, and through national language branches (Germany). International acceptance of the SETAC model continues with widespread interest in Russia and Africa. It is now the job of SETAC World Council to oversee the myriad SETAC activities around the world and to assure the integrity of the Society.

Environmental Toxicology and Chemistry, an internationally acclaimed scientific journal, has grown from a quarterly publication of fewer than 400 pages annually in 1982 to a monthly publication of 2,915 pages in 2001. Since January 2005, SETAC publishes a second scientific journal: Integrated Environmental Assessment and Management. IEAM is devoted to bridge the gap between scientific research and the use of science in decision making, regulation and environmental management. SETAC publishes the global newsletter SETAC Globe, peer-reviewed workshop and symposia proceedings, and a variety of technical reports.

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www.setac.org
Keynote speaker abstracts

KS01
The Biological Basis of Sustainability
Jeff McNeely
International Union for Conservation of Nature (IUCN)
Sustainability requires the capacity to adapt to changing conditions, and the ecosystems of our planet are the result of billions of years of successfully adapting to change. Their diversity provides today's societies with a wide range of services, ranging from provision of living resources to pollination and protection against natural hazards. In recent years, many industries are learning from natural systems, finding that "biomimicry" can help to solve many challenges in efficient and sustainable product design - everything from architecture that requires less energy to paint that washes itself. The "natural infrastructure" provided by nature continues to provide sustainable benefits to people, with no costs of construction or maintenance. The genetic diversity of plants will be essential for adapting to changing climates and meeting the increasing demands for food by a growing human population. And what does nature need in return? Being treated with respect, some sacrifices in opportunity costs, and being given full consideration - as critical elements in the strategies that governments and the private sector are developing to support sustainable development. The environmental foundations of sustainability will be essential for building the economic and social dimensions of future human wellbeing.

References
[1] UNEP (2011) Decoupling natural resource use and environmental impacts from economic growth, A Report of the Working Group on Decoupling to the International Water Council currently more than 60 percent - in arid regions even 90 percent - of water withdrawals is used for irrigation.


KS02
Sustainability - an ecological, economic and social programme for future development
Jochen Flasharth
President of the Federal Environment Agency, Dessau-Roßlau, Germany
The concept of sustainable development was introduced to the political and scientific debate 20 years ago at the UNCED conference in Rio de Janeiro. Since then we have seen, on the one hand, rapid growth of economies worldwide, increased resource consumption and apparent evidence of climate change. On the other hand there is growing awareness of public society that planetary boundaries exist and that the limited resources should be shared with developing countries and economies in transition. Technical and political concepts have been developed for how to save energy and resources, avoid toxic emissions and tackle major problems like rising energy demand, loss of fertile soils, lack of drinking water, demographic development, etc. 20 years after 1992, governments and stakeholders will meet again in Rio. What have we achieved? What should we achieve? And how can chemistry contribute to sustainable development?

Despite all the progress already achieved we need a fundamental change in the economy and lifestyle if we want future generations to be able to meet their needs. If business continues as usual, according to estimates by UNEP, energy-related CO2 emissions will increase continuously to more than 50 gigatonnes by 2030, although we need a reversal of emission trends if we want to comply with the 2°C target. The global demand for water will exceed supplies by 40 percent as early as 2030. According to the World Water Council currently more than 60 percent - in arid regions even 90 percent - of water withdrawals is used for irrigation.

The world economy needs to be changed in such a way that the limits of the global ecosystem are respected and irreversible damage is avoided. That means that we urgently need a greening of the economy which respects the planetary boundaries by setting environmental objectives. Key fields of action in industrialized countries include the transition to a low-carbon economy, reducing resource consumption in absolute terms and preserving biodiversity. The transformation to a green economy is a precondition for sustainable development and requires the contribution of the private sector in particular. Green economy is not an illusion of environmentalists which can only be realized in ecological niches. To the contrary, green economy offers major opportunities for business and employment and relates to the entire spectrum of economic activities. Various studies show that e.g. ambitious climate protection goals can create additional jobs, for example in developing renewable energies and in renovating buildings to enhance energy efficiency. Looking at the chemical sector it can also be demonstrated that eco-innovations offer win-win situations for companies active in that sector. However, a green economy will not develop on its own. In order to accelerate the transition process towards a green economy we need economic incentives for eco-innovations and sustainable consumption and production patterns. In industrialized countries, environmentally harmful subsidies need to be reduced and economic actors who behave unsustainably should bear the social costs they cause. We need a transformation from short-term to long-term responsibility as societies.

The chemical sector is one of the key sectors of Europe's economy. It is highly innovative and can therefore provide solutions to achieve the goals of sustainable development. In the past pollution by chemical industry was nearly synonymous with environmental degradation in general. Significant progress has been achieved in the meantime but problems and deficiencies still remain to be solved. One of the commitments made in Johannesburg in 2002 was that "chemicals should be used and produced in ways that lead to the minimization of significant adverse effects on human health and the environment". In Europe, REACH may provide safe chemicals but not necessarily sustainable chemicals. Complying with environmental laws is not sufficient to meet the requirements of energy and resource efficiency as well as sustainable production. Petrolieum is still the main feedstock for producing chemicals. The chemical industry is not sufficiently prepared to move towards renewable alternatives or - in the long-term - to hydrogen-based production.

The addressees of a sustainable chemicals policy do not only include the chemical industry. There must also be fundamental changes in agricultural practice in order to reduce the use of pesticides, veterinary pharmaceuticals and arable soil. Producing and using increasing amounts of chemicals, pesticides and pharmaceuticals is unsustainable in ecological, economic and social terms.

KS03
Resource Efficiency and Decoupling as Drivers for Sustainability
Mr. Arab Hoballah
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The remarkable economic and population growth of the 20th century was coupled to substantial increases in the extraction and consumption of natural resources, leading to increased negative environmental impacts. Total material extraction increased by a factor of about 8, and the price index of resources declined by about 30%, but many resources are now reaching their productive limits, as indicated by rising prices and lower grades of ores being mined.

However, economic growth globally was faster than growth of the rate of natural resources' consumption, and some negative environmental impacts have been reduced. Globally, about 25% less material input was required in 2002 compared to 1980 to produce one unit of real GDP which is a move towards a more resource efficient society. It appears that some 'dematerialization' of the world economy has occurred spontaneously. Accelerating this process of decoupling economic activity from consumption and environmental impacts is fundamental to future human well-being. Sustainability demands that resources be used more efficiently reducing economic and environmental costs of resource depletion and negative environmental impacts. Finally, while in some parts of the world local environmental impacts have declined, global impacts on the environment are becoming ever more important and severe, requiring an international policy response. Future policies will require a better understanding of the process, the so-called International Resource Panel (IRP) has undertaken to define the key issues and challenges, drawing on peer-reviewed research and international experiences [1]. In seeking to find where policy interventions for decoupling will be most effective, it is essential to consider the life cycle of resources, from initial extraction to ultimate disposal (or recycling) [2]. A major conclusion drawn by the IRP [1] is that decoupling is feasible, and is already happening, but further sustainability-oriented innovations are urgently required to enable decoupling to support sustainable development more effectively. More transformative change is required to meet the size of the challenge.

References
Special session abstracts

SS01-1
A Statement on the Sustainability of Global Human Societies: Toward a Declaration of the 6th SETAC World Congress, May 2012
L Kapustka
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As we develop the underpinnings for what will become the Berlin Declaration, we recognize the unique opportunity that SETAC has to contribute meaningfully to the global dialogue on sustainability. SETAC's tripartite structure of governmental, academic, and industry scientists and environmental professionals offers a model system for the trans-disciplinary development of a science of sustainability. Since its inception in 1979, SETAC's orientation has evolved from primarily environmental to include many aspects of social, environmental, and economic sciences, including decision analysis and policy analysis. SETAC enters the worldwide sustainability discussion in an interesting time. A society's ability to reach into any corner of the planet and produce a needed material from the ecological landscape (the rate at which the system supplies desired food, water, and other materials), but also from the services side (the assimilation rate of societal wastes).

As these challenges are discussed, a new paradigm is emerging, one that is different from the widely discussed pillars of sustainability. The new paradigm considers a nested relationship in which economics reside within societies, which in turn are wholly dependent upon surrounding ecological systems. This new perspective relates to the Millennium Ecosystem Assessment that highlights societies’ dependency on ecosystem services. SETAC’s challenge is to embrace and reinforce this science-informed conceptual model of sustainable - ecological landscapes, within this sustainability professional and society into one great sustainability statement. The Statement is building toward a Declaration that will offer a view of how the emerging science of sustainability can guide and inform societal decision-making processes.

SS01-2
Integrated Sustainability Assessment: Providing Scientific Support for Sustainability Policy
N. Pelletier, S. Sala', C. Cipau'a, European Commission, Joint Research Centre, Ispra, Italy
Sustainability is among the founding, long-term goals of the European Union and has been adopted as a guiding principle and objective for policy development by the European Commission. The EU Sustainable Development Strategy advances a vision for sustainable, prosperous communities predicated on resource efficiency, environmental protection and social cohesion. The Europe 2020 Strategy for ‘Smart, Sustainable and Inclusive Growth’ provides the policy agenda for sustainability.

The Sustainability Assessment Unit (Institute for Environment and Sustainability, DG Joint Research Centre) aims to advance science-based decision support for sustainability policy formulation, monitoring, and cross-cutting impact assessment. Towards this end, the Unit develops new methods and approaches for integrated sustainability assessment, including accounting frameworks and monitoring tools, reference data, and scenario modelling platforms to ultimately support policy making within the EU. The activities of the Unit are more and more targeted towards the implementation of objectives and policies foreseen within the Europe 2020 Strategy. This includes flagship initiatives such as ‘A Resource Efficient Europe’, the ‘Sustainable Consumption and Production and Sustainable Industrial Policy Action Plan’, the Strategy for ‘Innovating for Sustainable Growth: A Bioeconomy for Europe’, and the ‘The Raw Materials Initiative - Meeting our Critical Needs for Growth and Jobs in Europe’. Seeking to integrate and expand upon these initiatives, on-going development combines life cycle environmental, social and economic analyses within a common framework where indicator results can be assessed against sustainability targets and thresholds, or be used within a (land use) modelling platform to support the assessment of development policies via regionally resolved sustainability scenarios.

Life cycle thinking - one of the core elements of Unit activities - is implemented via the European Platform on LCA. In recognition of the pivotal importance of quality-assured data for robust assessments, the International Reference Life Cycle Data Network (ILCD) was created as a repository for sharing quality-compliant Life Cycle Inventory (emissions and resource use) data sets. It is open to all providers including business, national LCA projects, researchers, consultants, and others. Building on the ILCD, the Life Cycle-based Indicators and the European Environmental Footprint provide the methods and framework for life cycle assessments of environmental impacts related to European production and consumption.

SS01-3
Micronutrient to the rescue! How zinc fights a global health problem
E. Van Gendern
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Due to the current dependence on metals in society, the industry has focused its research programs on many attributes that contribute to the sustainable development of basic resources - food, water and energy. For essential metals, such as zinc, the linkage between soil and human deficiency has been identified as an area that requires significant attention in order to address childhood morbidity through improved agricultural productivity. For example, zinc deficiency is attributed to 800,000 deaths per year (450,000 deaths per year in children under the age of five). Further, it has been estimated that 50% of the world's agricultural soils are deficient in zinc. This high prevalence of zinc deficient soils in major agricultural zones limits crop productivity and lowers the nutritional value. In light of these critical and linked issues of zinc deficiency in soils, crops and humans, the International Zinc Association has launched programs that promote both short-term (food supplementation initiatives in conjunction with UNICEF) and long-term (improved crop production and nutrition through use of zinc containing fertilizers) strategies to address this global health problem. The presentation will provide an overview of these activities, including education outreach, policy efforts, communications and research.

SS01-4
Sustaining the Potential - making the most of nanotechnology
A. Davies
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Nanomaterials form an emerging and extremely promising technology, which already offers a wide range of economic and societal benefits and has the potential to unlock some of 21st Century society's biggest challenges. There are already encouraging indications that nanoscience can be an enabler to the environmental agenda, as it is in health care, electronics, construction, information technology and many other areas. However, to unlock this potential, Governments will need to bring science and policy development together, so that the benefits are maximised and the risks minimised. Developing partnerships will be key, as will the take-up of concepts such as responsible care; but all this needs to rest on a sound scientific evidence base, including life cycle analysis and risk assessment. This paper will explore these themes from the perspective of the United Kingdom and the European Union.

SS01-5
Sustainability communications: the do's and don'ts
J.F. Fava
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Organizations are seeking ways to continually describe their improved social, environmental and financial performance. Enterprise wide and product specific guidelines have been developed by a variety of groups and governments, e.g., the Global Reporting Initiative (GRI), International Organization for Standardization (ISO), DSM, FTC environmental claims guidance, and others. These efforts ensure a level playing field by advancing harmonized ‘rules’ on how an organization should communicate their story/progress without ‘green washing’. Additionally, with the increase in communication mechanisms, organizations are learning how to better utilize the internet and smart phones to reach broader audiences in new ways. This presentation will provide examples of good and ‘not so good’ sustainability communications and will conclude with general thoughts on what should be considered when developing and implementing a sustainability communications strategy.

SS02-1
Exposure assessment in ERA: from current tools to new approaches
A Di Guardo
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Environmental exposure can be assessed by means of monitoring activities and/or predictive approaches such as fate and bioaccumulation modelling. Current approaches in EU are mainly based on the use of models to estimate the fate of a chemical, as the Technical Guidance Document (TGD) dictates; however, these approaches, generally provide an average estimation of the fate of a chemical in a given environmental partitioning. These models do not take into account the spatial variability and can be currently adopted as a predictive tool for chemicals belonging to the classical “non polar” category, unless measured partition coefficients are provided. To overcome such issues, models should incorporate algorithm for predicting partitioning of, e.g., polar and ionized chemicals, and account for more spatial and temporal variability, considering the diversity of ecosystem exposure conditions. On the monitoring side, a number of environmental data and experimental parameters are needed to improve the modelling approaches: data from mixtures of chemical, (not commonly assessed as such), to the nanomaterials, for which a considerable lack of understanding of the fate still exists. Other knowledge gaps can be found in specific areas in which modelling approaches can be improved, from mass transfer parameters in specific environmental
SS02-2
Environmental effect assessment and risk characterisation of chemicals: what's wrong and how can it do better?
C. Jansen
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Ecological risk assessment (RA) procedures form the basis for the development of national and international regulations aimed at protecting the environment against potential adverse effects of chemicals. The currently used approaches are however, lack environmental realism which leads to high uncertainty and use of largely unsubstantiated 'safety factors'. This paper will review some major drawbacks of current environmental/ecological effects assessment methods and propose new approaches based on recent scientific developments.

SS02-3
Mechanistic effect modelling for ecological risk assessment: state-of-the-art, trends, and challenges
V Grimm
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Mechanistic effect modelling includes individual-level effect models, such as TK/TD and Dynamic Energy Budget models, and ecological models addressing populations and communities. Such models have the potential to overcome limitations of current risk assessment, in particular regarding higher tiers, complex exposure patterns, indirect ecological effects, and endpoints relevant for ecosystem vulnerability and services. The state-of-the art includes models that have been shown to be realistic enough to make robust predictions relevant for risk assessment of chemicals. However, so far such models had to be assessed on a case-by-case basis and often were not designed and communicated in a way that makes them suitable for regulatory decisions. Therefore, current initiatives are trying to make them suitably guidelines for Good Modelling Practice, for example the TRACE documentation framework developed in the EU-funded project CREAM. Main current challenges include: establishing acceptance criteria for mechanistic effect models, which then become part of Good Modelling Practice; agreement on standard species, scenarios, models, and ecological endpoints; installation of procedures that prevent standard models from becoming static and therefore being used incorrectly, and linking effect and exposure models. Mechanistic effects models are an indispensable tool for making risk assessment of chemicals more ecologically relevant. However, concerted actions, which correspond in scope and required effort to FOCUS, are needed to fully integrate such models in future regulatory risk assessments.

SS02-4
Addressing complexity in ecological systems - ignore it or embrace it?
V.E. Forbes
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Despite a widespread and long-held recognition that ecological systems are complex, traditional approaches to ecological risk assessment have largely attempted to ignore complexity. Instead, standard approaches rely on simplistic tests and assumptions with complexities glossed over by applying fixed ‘safety’ or ‘uncertainty’ factors. Advances in computer science, together with the development of appropriate modeling tools and modeling practice are facilitating more sophisticated and scientifically robust ways to deal with complexity. For example, extrapolation of effects across levels of biological organization, consideration of multiple stressors, and complex exposure scenarios can all be addressed using mechanistic individual-based models (ABMs). ABMs offer many advantages for ecological risk assessment; they are highly flexible, not limited by mathematical tractability, can incorporate spatial and temporal variability, can capture interactions among species and among multiple stressors, and are ideal for studying responses that cross levels of biological organization. There remain challenges for both the modeling and for model implementation so that the necessary complexities can be effectively addressed. The alternative, of course, is to continue to keep the complexities implicit and hope that by doing so will make them go away.

SS02-5
Priorities to improve ecological risk assessment for chemicals
T.C.M. Brock
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The ecosystem services concept can be applied to operationalize and harmonize the generic protection goals formulated in legislative documents. For each ecosystem service potentially affected by chemicals, important taxonomic groups need to be identified. It is key to identify “potential vulnerable” representatives (focal species) of these taxonomic groups. Different assessment tiers should focus from a risk manager cost-benefit point of view it may be necessary to develop criteria for a transparent spatial differentiation in specific protection goal options. Lack of a clear conceptual basis for the interface between the exposure and the effect assessment may lead to an overall low scientific quality of the risk assessment. This interface is defined as the Ecotoxicologically Relevant Concentration (ERC). Key is that the type of ERC used to express the “C” in the exposure estimates should not be in conflict with the ERC used to express the “C” in effect estimate. In the near future many of the ERC problems might be solved by using toxicokinetic / toxicodynamic or population models and exposure scenarios specifically developed for “vulnerable” focal species. The different tiers in ERA schemes need to be calibrated. An important lesson learned from the past is that the consistency of the tiered approach needs to be re-evaluated every time new chemicals (with a novel toxic mode-of-action) come on the market. An important pitfall in ERA remains the extrapolation of results of relatively simple model ecosystem experiments and computer simulation models to the diverse reality of the field. In relatively simple model ecosystem experiments and food-web models the indirect effects of chemical-stress observed may be a caricature of reality, since not all essential feedback mechanisms that may dampen temporal chemical-stress in natural ecosystems will be captured in the simple models. In the extended abstract more detailed information is given.

SS02-6
Regulatory point of view (focused on the implementation of REACH) on the new scientific challenges for ecological risk assessment on hazardous and PBT substances
I. Vaksa, J. V. Tarazona, B. Versonne
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Disclaimer: The authors are staff members of the European Chemicals Agency. The views expressed are solely the authors’ views and do not represent an official position of the Agency. The presentation will focus on the regulatory point of view (focused on implementation of REACH) on the new scientific challenges for ecological risk assessment in the case of hazardous and PBT substances which, as part of the registration process, require an environmental exposure assessment (EEA), the environmental risk characterisation, and/or the identification of operational conditions (OC) and/or risk management measures (RMM) for minimizing the emissions to the environment. The presentation will cover the following topics: - different roles and responsibilities under REACH involving ERA; risk characterisation/emission minimisation; substances registered that require an ERC and risk characterisation/emission minimisation; and the evaluation process: REACH-related regulatory difficulties related to EEA and risk characterisation/emission minimisation; - scientific challenges in EEA and risk characterisation/emission minimisation faced during dossier evaluation so far; major EEA and risk characterisation/emission minimisation related deficiencies in dossiers evaluated so far: current experience, further needs and possible solutions; relevance of the identified challenges and proposed recommendations in the draft SCCS/SCHER/SCENIHR opinion regarding the implementation of the REACH Regulation; further suggestions and research needs in the regulatory scientific context.

SS02-7
The view of a regulator on the possibilities for applying more ecologically relevant effect assessments
H.C. Clausen
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The presentation will address different approaches to effects assessment and risk characterisation with the view of a regulator focusing especially on the practicability, seek simplicity, and distrust it. The simpler the system, the easier it is to handle, and it is generally more transparent. The more complex the testing and modelling is the greater the uncertainty, and distrust it. The simpler the system, the easier it is to handle, and it is generally more transparent. The more complex the testing and modelling is the greater the uncertainty, and distrust it. The simpler the system, the easier it is to handle, and it is generally more transparent. The more complex the testing and modelling is the greater the uncertainty, and distrust it. The simpler the system, the easier it is to handle, and it is generally more transparent.

SS02-8
Addressing the new challenges for risk assessment: An industry perspective
P. Campbell
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This is a comprehensive overview of ecological risk assessment approaches and the high level goals of this paper, such as reduced animal testing and development and promotion of non-animal methods, and high level ecological risk assessment expertise, should be commended. Key recommendations such as: adopting higher-tier ecological risk assessment tools; a list of requirements for new innovative approaches before they can be used in regulatory ecological risk assessments; and the need for further research on how to deal with multiple stressors are also well. More one holistic challenges for this document, is the breadth of remit. Currently it encompasses both prospective and retrospective regulatory efforts (addressing alternate types of potential chemical contaminants (general chemicals), where the issues and the available data sets are going to be very different. Therefore, one way to improve this document would be to consider restructuring the content to provide clear and specific advice to distinctly different situations eg. Ecological Risk Assessment for Chemical Registration and Site Specific Ecological Risk Assessment, being 2 potential examples. One of the ecological risk assessment challenges for the pesticide industry is extrapolation of risk assessment conclusions eg between species, different environmental conditions and geographical regions. One of the tools recommended within this document to help with this issue is the expanded use of modelling, and again this is justified. However, another recommendation is for greater uncertainty analysis and application of appropriate uncertainty factor. The industry experience with this option is not so helpful eg Bird & Mammal Guidance Document, SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
where low toxicity herbicides are still failing the risk assessment. In addition, the current practice of application of safety factors to the results of Mesocosm/pond studies now there is no regulatory benefit to carrying out such studies. Consequently, a real opportunity is now missed to test, observe and understand potential in-direct and community level aquatic effects, both of which are highlighted as an area where more information is required in this document. The key issue here is that there will always be uncertainty in ecological risk assessment and therefore we can really reduce the need for expert judgement as recommended, without having to continue to apply large over protective uncertainty factors.

**SS02-9**

**Environmental Risk Assessment - Basic principles, future trends.** View from industry employed environmental scientist on the EU (DG Sanco) document ‘Addressing the new challenges for risk assessment’

P. Dohmen

BASF SE, LIMBURGERHOF, Germany

The current EU document represents the state of the art in environmental risk assessment (ERA) including new trends and combining high level environmental protection and realism. The ecosystem services concept offers a suitable approach to determine which impact may be tolerable where and when. Established ERA uses a stepwise approach starting with standardized studies combined with appropriate AF and where needed followed by higher tier assessments and respective adjusted AF. The new EU document follows this approach and presents additional refinements in order to improve the realism of risk assessment. This is certainly desirable; however, the additional data must not become requirements. The document describes when to ask for additional information or call for specific risk management actions; i.e. analyse data, include modelling etc. This should be verified by respective field investigations and/or monitoring. Only then specific action should be warranted. This general approach is strongly supported. The document refers to ‘indirect effects’. These occur as a consequence of direct effects. If there is low risk of direct effects, then indirect impacts are of low relevance. Only when direct effects are considered acceptable, more consideration should be given to indirect effects. Site specific evaluation will improve realistic ERA. However, this may require additional information and will not be met by additional costs. As nanomaterials and engineered nanoparticles are additional sources of concern, they are not addressed in this document. These nanomaterials represent a promising tool capable to combine taxonomically based assessments with functionally based assessment (more significant in ecological terms). This is valid and relevant; however, this tool is only useful in the regulatory context if it is avoided that individual results from single species will override the trait-based approach. Modelling has been included in ERA allowing to extrapolate risk allowing without the need of excessive (animal) testing; it will also address uncertainties more quantitatively. This should be done in an overall realistic assessment; simply adding up various individual uncertainties has nothing to do with a realistic ERA.

**SS03-1**

**Nanotechnology: Peeking into the Future**

S.J. Marshall

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There is great potential for nanotechnology to be applied in the broad range of societal sectors. New applications and product formats are likely to add to current emissions into the environment, e.g., via consumer disposal of domestic products down the drain or via direct emission to, or use in, the environment. Given the developing state of definitions and of characterisation and risk assessment methods, industry is faced with uncertainty over the likely success of new innovation involving nanomaterials and technologies. Rapid progress is needed in these assessment methodologies in order to establish guidance on data requirements and how they should be interpreted for risk-based decisions. In the meantime communication with stakeholders needs particular care to ensure that both research and safety related data can be considered in context of the risks to man and the environment. This presentation will elaborate on developments from an industry perspective.

**SS03-2**

**The regulatory perspective on engineered nanomaterials**

B. Sculli-Klutter1, M. J. Dov2, T. F. Fernandes3

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As nanomaterials are used in an increasing range of applications there is growing debate regarding their safe use and possible impact on human health and the environment. There is no specific legislation in the European Union (EU) or the US on nanotechnology or nanomaterials, but legislation on worker protection, environmental legislation, chemical legislation, specific products legislation etc. apply in principle to nanomaterials. In the EU, nanomaterials are covered by the chemicals legislation, REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), which addresses chemical substances, in whatever size, shape or physical state, although there are no specific provisions for nanomaterials in REACH. Any introduction of provisions specific to nanomaterials requires the adoption of a definition of nanomaterial. Although in October 2011 the EU Commission adopted the Recommendation on the definition of a nanomaterial, at this stage this does not constitute as yet enforceable legislation. In the USA, nanomaterials are primarily covered by the industrial chemicals legislation TSCA (Toxic Substances Control Act), pesticides legislation FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act) and for foods and drugs, the FDCA (Federal Food, Drug, and Cosmetic Act). In the USA, before a new chemical is commercialised a manufacturer or importer must submit a new chemical notification and a determination must be made that it may not present an unreasonable risk to human health or the environment. This presentation will give an overview of the current regulatory perspectives for engineered nanomaterials including a short summary concerning available guidance on safety/risk assessment, and will briefly cover additional international activities.

**SS03-3**

**Advances in methods for analysing nanoparticles in complex environmental media**

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The analysis of engineered nanomaterials (ENMs) in the environment is still a challenging task. Advances in the study of the environmental fate, transport, and ecotoxicological effects of ENMs have been hampered by a lack of adequate techniques for the detection and quantification at environmentally relevant concentrations in complex media. The analysis of ENMs differs from traditional chemical analysis because both chemical and physical forms must be considered. Because ENMs are present as colloidal systems, their appearance and physicochemical properties are dependent on the surrounding conditions and may be of transient character. By trying to observe, isolate, and quantify ENMs their physicochemical properties may be changed, making the analysis extremely susceptible to artifacts. The most pressing research needs are the development of techniques for sample storage, extraction/separation, and cleanup that introduce minimal artifacts; to increase the speed, sensitivity, and specificity of analytical techniques, as well as the development of techniques that can differentiate between abundant, naturally occurring particles, and manufactured nanoparticles. The direct and non-invasive detection of ENPs in complex samples is hampered by the presence of sample components interacting with the ENPs and/or interfering with the analytical technique. Separation and isolation of the ENPs is therefore necessary in most cases. The basic idea of most currently developed methods is to provide particle sizing capabilities by the use of high spatial resolution or chromatography-like separation techniques. These core capabilities are then combined with material, element- or mass-specific detection. Especially high resolution X-ray, Field-Flow Fractionation and single-particle ICP-MS based techniques have made considerable progress because of their particle size and compositional selectivity. The presentation will cover examples of particle/matrix combinations and discuss the pros and cons of the methodological approaches. It will also address in detail the challenges posed to analytical methods arising from the definition of Nanomaterials issued by the EU commission.

**SS03-4**

**Fate and behaviour of engineered nanoparticles affecting exposure in natural systems**

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Research over the past decade has provided a fundamental understanding of the behaviour of engineered nanomaterials in surface waters, sediments and soils. We are well aware that aggregation is an important pathway for most nanoparticles, while for some, e.g., ZnO dissolution has been shown to be an important control on exposure. This transformation will depend both on the physical and chemical properties of the nanoparticle, such as size, surface charge and the presence of coatings, and of the receiving environment, such as ionic strength, pH, and its chemical composition including both dissolved and colloidal organic and inorganic components. Because of the difficulty in working with what could be termed ‘environmental concentrations’ of nanoparticles, studies of their fate and toxicity have for the most part been undertaken at parts per million concentrations where both the chemical and physicochemical measurements are more reliable, and usually in controlled synthetic water, sediments and soil matrices. Interactions with organic matter have usually been studied using additions of “standard” humic acids. More recently, studies with natural waters, sediments and soils have been undertaken with the aim of investigating the fate and behaviour of nanoparticles in complex, naturally occurring systems. Here their fate and potential toxicity can dramatically change as a result of aggregation of nanoparticles with natural mineral and organic colloids (heteroaggregation) whose concentrations are likely to exceed those of the nanoparticles, as distinct from homoagregation with the same nanoparticles. Heteroaggregation will change the surface charge and mobility of nanoparticles and tends on the low bioavailability of natural colloids, is likely to reduce the toxicity of nanoparticles. Such interactions also occur during wastewater treatment (as well chemical transformations, e.g., sulfidation of silver nanoparticles) and will likely contribute to the ultimate transfer of nanoparticles to sediments or to their immobilisation on the soil solid phase. Examples will be presented. The challenge will be to link the exposure concentrations determined under these more realistic environmental scenarios to bioaccumulation and potential toxicity, so that risk assessments and life cycle models can provide more reliable predictions for environmental managers.
**SS03-5**

Challenges in determining the ecotoxicological responses of engineered nanomaterials

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The ecotoxicology tool box contains a myriad of different bioassays and toxicity tests that use a variety of biological end points. These responses are from different levels of biological organisation, from molecular level to organisms, which can be extrapolated to imply population level effects. However, while there is general agreement that many of the existing regulatory test methods may be adapted or modified to work with nanomaterials, less attention has been given to the underlying biological responses. This paper aims to critically evaluate the scientific rationale behind the selection of biological responses as end points for ecotoxicity tests with nanomaterials, and asks whether or not all the important biological responses have been considered in the testing strategy. Ideally, a mechanistic explanation should associate any given biological response with a mechanism, but this understanding is lacking for many materials and organisms. Biological responses are time-dependent and for many materials the sequence of exposure, bioavailability, uptake kinetics, and the onset of the biological response has not been verified. An association between the presence of the nanomaterial in the tissue and the biological response (the target organ approach) is not the only paradigm to consider, with secondary toxicity and latent effects also possible. Evidence is emerging that some of the traditional end points in regulatory tests, such as growth or survival, may not be the most sensitive measurements for nanomaterials. However, alternative approaches, combining the signatures of global changes that provide on the ecosystem services, rivers and streams, as well as their interactions. These WFDs deal with data collection (WFD1), hydrology (WFD2), sediment transport and river channel morphology (WFD3), chemical and biological quality (WFD4), ecosystem processes (WFD5), modelling (WFD6), socioeconomic scenarios (WFD7), ecosystem services (WFD8), river management (WFD9) and coordination (WFD10).

**SS03-6**

The expansion of agricultural frontiers and its consequences on the environment. The case of Argentina during last decades

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Argentina is one of the world’s major agricultural producers and exporters of proteins and other food commodities. In 2006, Argentina was the second largest exporter of agricultural products and one of the world’s main cereal exporters, becoming a major player in the world market and a key supplier of commodities from Latin American Countries (LAC). The main goal of this introductory speech is raising issues on the environmental cost that this increased production is causing in comparison with economical benefits arising from the exportation of food commodities from LAC.

**SS03-7**

**THE SCARCE CONSOLIDER PROJECT ON WATER SCARCITY IN IBERIAN RIVER BASINS**

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Water resources in Spain are subjected to rising pressures, related to the socioeconomic activities of an increasing human population, expressed by accelerated land use changes, and the specific climate characteristic of Mediterranean countries. The main panels on climate change predict a future scenario of increasing frequency of floods and extended droughts in the Iberian Peninsula, mostly in the Mediterranean basin. This will be added to the already existing problems, and will probably affect the available water resources, their quality, the functioning of associated ecosystems, especially the gaseous exchangers in and between the surface and subsurface environments and the aquatic ones. Therefore, the project has assembled a multidisciplinary team of leading scientists in the fields of hydrology, geomorphology, meteorology, chemistry, ecology, ecotoxicology, economy, engineering and modelling, in an unknown effort in the CONSOLID-ER framework. The project also considers the active involvement of Water Authorities and other relevant agents as stakeholders. SCARCE has two complementary objectives. The first tackles basic research questions and will define the long-term patterns and the mechanisms that operate in the hydrology, water quality, habitat dynamics, and ecosystem structure and function of Mediterranean basins. The second objective is related to the effects of climate and human footprint on the emergence of scenarios of global change that provide on the ecosystem services, rivers and streams, as well as the urgent need to implement and eventually refine the water management policies demanded by the EU Water Framework Directive (WFD). Therefore, the project emphasizes linking basic research and management practices in a single framework. The project has the external support of several Water Authorities and stakeholders. SCARCE is structured across a series of Horizontal and Thematic Work Packages that are the vehicle for the implementation of these, as well as their interactions. These WFDs deal with data collection (WFD1), hydrology (WFD2), sediment transport and river channel morphology (WFD3), chemical and biological quality (WFD4), ecosystem processes (WFD5), modelling (WFD6), socioeconomic scenarios (WFD7), ecosystem services (WFD8), river management (WFD9) and coordination (WFD10).

**SS03-8**

Emerging organic contaminants in food: a global sustainability challenge

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The world population is growing faster than in the last century. In particular, food commodity has been increased to satisfy increasing demands of their populations, resulting in a less controlled growth of food production, particularly food commodities. This higher demand of commodities has been partially satisfied by improving the production technology but also increasing cultivated areas, which were the in use of areas previously occupied by wild biota. Modern agricultural practices make intensive use of agrochemicals to warrant both productivity and health of cultivars. On the other hand, cattle production uses veterinary drugs, accelerated grow systems (feed lot and similar), sometimes using non authorized anabolic products. Within this framework, South America (SA) is probably the area showing the highest and fastest increase in food productions, which result in almost uncontrolled augment of areas dedicated to the production of soya and crops. Particularly Brazil and Argentina have been rapidly increased their national incomes arising from the exportation of food commodities, in addition to a growing production of bio-fuels. The increase of international prices for soya and maize have been the main argument used for small and big farmers to increase the cultivated area, affecting native forest, spreading agrochemicals in the proximity of cities, controlling pests and weeds but also affecting non target organisms. During this short introduction some figures on the growing demand on food commodities, as stated by FAO (UN) will be presented, with particular emphasis in the production of Latin American Countries (LAC). The main goal of this introductory speech is raising issues on the environmental cost that this increased production is causing in comparison with economical benefits arising from the exportation of food commodities from LAC.
show substantial discrepancy between field observations in polluted environments and predictions derived from laboratory bioess.

What is the role of redundancy (which appears to be specially pronounced in soils)? I will summarize theoretical approaches, scrutinize some frequently used endpoints and there an ideal species number, community structure? How do we adequately compare soils with different climates, different keystone species? How do we measure resilience? Among the most prominent groups of emerging food contaminants can be considered industrial origin contaminants as perfluorinated compounds (PFCs), polybrominated bi-

Predicting or measuring exposure and bioavailability of contaminants in soils has progressed significantly in the last 10 years. Regulators dealing with the prevention and remediation of contaminated soils are aimed at the soil as an environmental compartment (soil protection act), industries (environmental regulations), chemicals (REACH, pesticide regulations, Nitrate directive) or waste management (Waste Directive). Policies to prevent and remediate soil contamination use prognostic and diagnostic instruments. Prognostic instruments include soil quality standards and predictions of environmental concentrations of chemicals to manage (intentional) emissions of contaminants. Site specific ecological risk assessment (ERA) is a diagnostic instrument that also uses quality standards, such as intervention values, combined with other lines of evidence. Since the 90-ties species sensitivity distributions (SSD) have been increasingly used in prognostic and diagnostic instruments, to determine e.g. safe levels and intervention values for contaminants. SSD’s seem less suitale for contaminants that have a very specific mode of action, essential nutrients. Some prognostic instruments, for instance for pesticides, may include higher tier testing in case predicted environmental concentrations exceed safe levels. More lines of evidence in site specific ERA may include observations from mesocosms and field tests, as well as, ecological knowledge on the role and function of species in the environment. A Triad approach is often recommended, in which chemical, toxicological and ecological data for a contaminated site are assessed along converting lines of evidence. The European Soil Strategy (2006) has identified 7 additional soil threats, besides contamination. These threats refer also to, biological and physical aspects of soil. The Soil Strategy stresses the importance of soil functions by explicitly listing these as goals, as protection goals. The ‘soil functions’ largely comply with the definition of ecosystem services, a concept that gains an increasingly central role in environmental assessments. The potential of this concept for assessing soil quality will be briefly addressed.

Historical and regulatory background of soil quality assessment
J. van Wensem
Soil Protection Technical Committee (TCB), The Hague, Nederland
From the 70-ties of the past century onwards concern has been raised about levels of contaminants in the environment. Main sources of contamination are industrial processes, waste disposal and intentionally applied chemicals such as fertilizers, pesticides and veterinary drugs. Regulations dealing with the prevention and remediation of contaminated soils are aimed at the soil as an environmental compartment (soil protection act), industries (environmental regulations), chemicals (REACH, pesticide regulations, Nitrate directive) or waste management (Waste Directive). Policies to prevent and remediate soil contamination use prognostic and diagnostic instruments. Prognostic instruments include soil quality standards and predictions of environmental concentrations of chemicals to manage (intentional) emissions of contaminants. Site specific ecological risk assessment (ERA) is a diagnostic instrument that also uses quality standards, such as intervention values, combined with other lines of evidence. Since the 90-ties species sensitivity distributions (SSD) have been increasingly used in prognostic and diagnostic instruments, to determine e.g. safe levels and intervention values for contaminants. SSD’s seem less suitable for contaminants that have a very specific mode of action, essential nutrients. Some prognostic instruments, for instance for pesticides, may include higher tier testing in case predicted environmental concentrations exceed safe levels. More lines of evidence in site specific ERA may include observations from mesocosms and field tests, as well as, ecological knowledge on the role and function of species in the environment. A Triad approach is often recommended, in which chemical, toxicological and ecological data for a contaminated site are assessed along converting lines of evidence. The European Soil Strategy (2006) has identified 7 additional soil threats, besides contamination. These threats refer also to, biological and physical aspects of soil. The Soil Strategy stresses the importance of soil functions by explicitly listing these as goals, as protection goals. The ‘soil functions’ largely comply with the definition of ecosystem services, a concept that gains an increasingly central role in environmental assessments. The potential of this concept for assessing soil quality will be briefly addressed.

Soil ecology and soil quality relationships
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Whenever dealing with environmental hazards, quality assessment of the respective compartment has been heatedly debated. Buzzwords and concepts have repeatedly changed names (e.g. critical loads, critical thresholds, tipping points), yet the key issue - what is a healthy system (here: soil)? - has never been thoroughly conceptualised. Is there an ideal species number, community structure? How do we adequately compare soils with different climates, different keystone species? How do we measure resilience? What is the ecological redundancy (which appears to be specially pronounced in soils)? I will summarise theoretical approaches, scrutinise some frequently used endpoints and quality measures such as BQSQ or PICT and discuss their pros and con’s. With respect to available and desirable data I will particularly stress the relevance of various test systems and endpoints, short-term versus long-term monitoring data as well as natural stressors. Trying to structure everything in a logical way and wrapping it with my own considerations, I hope being able to give some new insights for soil quality assessment.

Predicting or measuring exposure and (bio)availability assessment
M. J. Smith
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Predicting or measuring exposure and bioavailability of contaminants in soils has progressed significantly in the last 10 years. For metals, robust methods to determine speciation of metals in both the solid phase and in soil porewater are now available. We also know that toxicity of metals is moderated by competitive ion effects at the receptor surface, and these interactions as well as metal partitioning between solid and solution phases can be predicted and/or modelled. “Bottom up” or mechanistic approaches to predict metal toxicity across a wide range of soils have found less success in regulatory implementation than “top down” or empirical methods, largely because of the simplicity of data requirements and ease of calibration and validation of the latter. It is important that the degree of complexity and data requirements of frameworks needed for Tier 1 risk assessments or contaminant screening values be borne in mind during development. Direct measurement of contaminant bioavailability is often regarded as a competitor to predictive models, but it should be realised these are best suited to different levels of the risk assessment process. Great improvements in measures of metal fate, exposure, and bioavailability in soils are now possible with advances in instrumentation and techniques that often now allow rapid and/or in situ measurements that will greatly enhance not only risk assessments but also could assist with risk management processes (remediation).

Natural stressors in ecotoxicological studies and ERA: do they matter?
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Although already some 20 years ago scientists were warning that ecological risk assessment (ERA) based on simple (eco)toxicological essays may be seriously biased, this is still the most commonly used methodology. Although the need for chronic and sub-chronic tests has been recognized to some extent in recent years, what certainly improved the confidence in ERA, these tests are also performed under standard constant laboratory conditions. Moreover, even if these conditions are usually called “optimal”, this is not necessarily true. First of all, however, organisms are exposed to a whole range of suboptimal conditions in their natural environment, and these conditions are perpetually fluctuating. Organisms are, thus, exposed to a number of different stress factors which, irrespective of any possible pollution, affect individuals and populations. The important question from the ecotoxicological point of view is whether these non-chemical stressors can significantly bias the predictions of ERA. In recent years the number of studies showing significant and environmentally relevant interactions between chemical and non-chemical stressors have increased substantially. For soil environment, temperature, drought, food supply, and pathogen are all potential stress factors. The complexity of these stress factors varies from site to site, and on a macro-scale even more complicated and difficult to study. Despite these difficulties, it seems that neglecting the interactions cannot be justified any longer. This is proved not only by laboratory studies indicating on significant interactions, which always can be questioned from the point of view of their relevance to ERA, but also by field surveys which can show substantial discrepancy between field observations in polluted environments and predictions derived from laboratory bioassays. The authors of a recent paper devoted to this problem concluded that “understanding the effects of toxic agents in a complex environmental gradient may require more research on the interaction between toxicity.
SS06-5
Microbial, single species, and higher tier test for predictive assessment of chemical effects on soil quality
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TRIAD, is used site-specific on PAH- and heavy metal contaminated sites in Denmark. The various aspects of the TRIAD approach are based on a set of chemical-, toxicological- and ecology related data collected among others in the EU project “Development of a decision support system for sustainable management of contaminated land by linking bioavailability, ecological risk and ground water pollution of organic pollutants” or in short “LIBERA”.

SS06-6
Can we realize tangible benefits from using complex ‘omics tools when assessing soil quality?
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SS06-8
Case study 1: prognostic - risk assessment of plant protection products
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SS06-9
Site-Specific ecological risk assessment. Case-study 2
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SS07-1
The decline of the honeybee: a modelling approach
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SS07-2
Using population models to determine the impact of herbicides on endangered species: an example with lange's metalmark butterflies at the Antioch Dunes National Wildlife Refuge, California
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SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
The importance of absorption, elimination and feeding pattern: using toxicokinetics modelling to refine the risk assessment of pesticides to wildlife

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Current risk assessment for mammals is based on external exposure measurements, but it has long been acknowledged that effects of toxicants are better correlated with systemic availability, which depends on many factors, e.g., bioavailability (fraction of dose reaching systemic circulation or site of physiological activity), absorption, distribution, metabolism and excretion (ADME). Toxicokinetic (TK) models are mathematical descriptions of these processes and can be used to refine risk assessments. In toxicological studies of pesticides and biocides, little internal dose data are routinely generated, and the use of toxicokinetic models in risk assessment for pesticides is relatively new. Moreover, for risk assessment of pesticides, the toxicokinetic (and toxicodynamic) should be interpreted in the context of potential exposure in the field. Therefore, it is key to include different timescales of exposure and behavioural factors such as feeding pattern in study. Here, we present a case study for an insecticide. A toxicokinetic model was parameterised using data from a rat study with 14C-labelled material. The number of compartments (tissues) in the model and the complexity of the processes of absorption and elimination were investigated. Feeding pattern and avoidance of contaminated food were included in the model as they influence internal concentration and hence risk in natural environments. High variability in kinetic parameters between individuals meant it was necessary to test the effects of different combinations of values for absorption ka and elimination ke rate constants on concentration in the bloodstream. This was done for different scenarios, e.g., LD50 eaten as a bolus dose and more natural feeding patterns. Maximum internal concentrations (CMaxBody) were compared across scenarios. Our simulations showed that, irrespective of ka and ke combinations, higher CMaxBody were reached when the LD50 was given as a bolus dose than when realistic worse-case scenarios were used. Nevertheless, the difference between CMaxBody reached after a bolus dose or feeding naturally depended on which combination of ka and ke was used; the lower the ka and ke the closer the feeding body burden came to the bolus dose body burden. Ongoing modelling is underway and the impact of avoidance mechanism on the insecticide body burden will be reported. The possible use of body burden model as a refinement option in bird and mammal risk assessments will be discussed.

Mechanistic modeling of the effects of perchlorate on the thyroid gland and risk to post-natal developing mammals

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Mechanistic models can be used to predict the risk to mammal populations from exposure to perchlorate. We developed a physiologically-based toxicokinetic (PBTK) model with diffusion mechanisms governing the uptake and distribution of perchlorate. Compartmental models include blood plasma, liver, kidneys, gut wall, gut contents, and thyroid and pituitary endocrine glands. The thyroid model describes the binding, distribution, and disposal of thyroxine (T4) and triiodothyronine (T3) including homeostasis mechanisms. These thyroid hormones inhibit the secretion of TSH by the pituitary, which stimulates secretion of the thyroid hormones. Health risk to mammalian species depends upon the effect of perchlorate on thyroid percolate in consumed food and water. Prenatal and postnatal development may be impacted through perchlorate transfer by the placenta and during lactation. Non-lethal assessment endpoints, such as growth retardation and offspring deformities, define health risk. Several individuals are simulated to obtain population level effects. The model is stochastic, with random variables for residue in water and food items.

Why complexity matters: using ALMaSS for risk assessment of wildlife

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The focus of occupational risk assessment (ERA) for non-target organisms is on the direct effect of the toxicant. In some cases what are often termed large-scale sources of variation are also considered (e.g., expected crop distribution). However, these describe only part of the variation that occurs in the real world. Landscapes vary in structure, meaning that the field size and proximity to primary NTO habitats will vary. There is climatic variation driving changes in phenology and behaviour, and management changes in the proportion of crops grown, but also how they are cultivated in time, and all of these factors can affect the risk assessment. There is also another, different, phenomenon: the property of real-world systems is that they are inherently dynamic and where multi-scale influences are integrated. This was done for different scenarios, e.g., LD50 eaten as a bolus dose and more natural feeding patterns. Maximum internal concentrations (CMaxBody) were compared across scenarios. Our simulations showed that, irrespective of ka and ke combinations, higher CMaxBody were reached when the LD50 was given as a bolus dose than when realistic worse-case scenarios were used. Nevertheless, the difference between CMaxBody reached after a bolus dose or feeding naturally depended on which combination of ka and ke was used; the lower the ka and ke the closer the feeding body burden came to the bolus dose body burden. Ongoing modelling is underway and the impact of avoidance mechanism on the insecticide body burden will be reported. The possible use of body burden model as a refinement option in bird and mammal risk assessments will be discussed.

Incorporating environmental complexity in assessing chemical risks for soil organisms using mechanistic effect models

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Unlike larger terrestrial vertebrates, such as birds and mammals, most soil organisms have limited mobility and may not as easily be able to seek shelter from environmental conditions such as temperature and solar radiation. Here, we present a case study for an insecticide. A toxicokinetic model was parameterised using data from a rat study with 14C-labelled material. The number of compartments (tissues) in the model and the complexity of the processes of absorption and elimination were investigated. Feeding pattern and avoidance of contaminated food were included in the model as they influence internal concentration and hence risk in natural environments. High variability in kinetic parameters between individuals meant it was necessary to test the effects of different combinations of values for absorption ka and elimination ke rate constants on concentration in the bloodstream. This was done for different scenarios, e.g., LD50 eaten as a bolus dose and more natural feeding patterns. Maximum internal concentrations (CMaxBody) were compared across scenarios. Our simulations showed that, irrespective of ka and ke combinations, higher CMaxBody were reached when the LD50 was given as a bolus dose than when realistic worse-case scenarios were used. Nevertheless, the difference between CMaxBody reached after a bolus dose or feeding naturally depended on which combination of ka and ke was used; the lower the ka and ke the closer the feeding body burden came to the bolus dose body burden. Ongoing modelling is underway and the impact of avoidance mechanism on the insecticide body burden will be reported. The possible use of body burden model as a refinement option in bird and mammal risk assessments will be discussed.

2008-1

Combined Effects of Climate Change and Contaminants on Arctic Ecosystems and Humans

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The Arctic Monitoring and Assessment Programme (AMAP) has produced several scientific assessments documenting the levels, trends and effects of specific groups of contaminants such as heavy metals, persistent organic pollutants (POPs), and radionuclides, and the status, trends and effects due to climate change on Arctic ecosystems and humans. The difficulty of attributing the effects observed to a specific problem or cause triggered an increasing need to analyse the potential combined effects of several contaminants in mixture and also together with the current observed Arctic climate change. Based on this, the project 'Combined Effects of Climate Change and Contaminants in the Arctic' was initiated in 2005. The project has focused on the combined effects of climate change, POPs, mercury and radionuclides in selected parts of the Arctic/sub-Arctic marine and terrestrial food chains and humans. The project was carried out by eight Nordic research groups. The first phase of this project has been completed and an overview of the results is presented here. The research priorities covered the following topics: 1) A theoretical analysis of the effects of climate change on the long-distance atmospheric transport and deposition of several selected POPs and mercury in the Arctic using a 3-D atmospheric chemistry-transport model; 2) A modeling study of POPs in snow and snow in air from Svalbard and Greenland to study the concentrations and mobility of these contaminants in relation to environmental conditions such as temperature and solar radiation; 3) A modeling study in which the effect of climate change, in terms of increased temperature and primary production, on the partitioning and bioaccumulation of organic contaminants in biota at various trophic levels in the Arctic marine environment was investigated and quantified; 4) An analysis of changes in the fallout of radionuclide (137Cs) and mobility in the Arctic frozen/melt terrestrial ecosystems and its uptake in terrestrial food webs; 5) A review of abiotic and biological processes relevant to the concentrations and distribution of mercury in the Arctic environment and bioaccumulation in biota;
6) An analysis of the concentrations of selected POPs and mercury in the blood of several indigenous peoples populations in Arctic Russia as a prelude to a further study of their potential effects on people living mainly on local marine and terrestrial food sources.

SS08-2 Southern Ocean and the Antarctic climate system
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The planetary climatic system is sensitive to the conditions of energy exchange between the ocean and the atmosphere in the Southern Ocean. The interbasin exchange accomplished by the Antarctic Circumpolar Current (ACC) is an important link in the global overturning circulation and also admits anomalies formed in one basin be carried around the globe to influence climate in remote locations. As a result, the Southern Ocean strongly influences climate patterns and the cycling of carbon and nutrients. A reliable knowledge of the quantitative characteristics of energy, mass and gas exchange appears to be a basis for the estimation of possible climatic changes in the near decades. Recent observations suggest the Southern Ocean is indeed changing: Data from Argos floats and research vessels confirm that the Southern Ocean, particularly the southern flank of the ACC, has warmed more rapidly than the global ocean as a whole. The average anomalies for 2005-2011, i.e., water temperature deviations from the mean values in the Southern Ocean turned out to be positive in the upper 1500 m layer with a maximum of +0.2 °C in the layer between 250 and 450 m. The average for the same seven years salinity deviations from mean values indicate weak water freshening in the Southern Ocean with the largest negative deviations of about -0.04 °C achieved at 200 m depth. Black carbon (BC) particles are products of incomplete combustion. BC is the most efficient aerosol specie absorbing visible light and it exerts a warming effect in the atmosphere. The significance of BC is higher in the Arctic than elsewhere. Its atmospheric light absorption is enhanced by the high albedo of snow and ice surfaces in snow covered areas, and the deposited BC particles in snow and ice reduce the surface albedo and accelerate the melt rate. BC has a relatively short lifetime in the atmosphere, in the order of days, which implies that emission reductions of BC rich sources would lead to fast responses in climate impacts. However, since it is emitted along with other particulate and gaseous species that have multiple cooling and warming effects, the net-effect of all relevant species should be taken into account when designing emission mitigation strategies. Recent assessments indicate that a set of measures targeting BC could reduce the projected Arctic climate impacts significantly. The reductions would also bring about co-benefits for public health.

SS08-3 Persistent Organic Pollutants in Antarctica; System Input from Distant and Local Contaminant Sources
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Persistent Organic Pollutants (POPs) are ubiquitous toxic compounds that are incorporated into food chains with high efficiency. Polar Regions have long been established as receiving environments for POPs. In order to manage environmental contamination by POPs in Antarctica, information regarding system input to this remote region is required. Here we present the first results of atmospheric input of POPs to the Australian Antarctic Territory in over a decade. Further, we present the first audit of an Australian research base as a local emitter of newly listed POPs and explain biota accumulation of PCFs in terms of species foraging ecology and the dynamics of the Antarctic circumpolar current. It has recently been shown that climate change is beginning to mobilise global POP reservoirs. Our findings therefore also provide a baseline for temporal monitoring of how this remote region stands to be impacted as global secondary sources are perturbed.

SS08-4 Impacts of black carbon on Arctic climate
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Black carbon (BC) particles are products of incomplete combustion. Major emission sources globally are transport and residential heating and cooking as well as wildfires. BC is the most efficient aerosol specie absorbing visible light and it exerts a warming effect in the atmosphere. The significance of BC is higher in the Arctic than elsewhere. Its atmospheric light absorption is enhanced by the high albedo of snow and ice surfaces in snow covered areas, and the deposited BC particles in snow and ice reduce the surface albedo and accelerate the melt rate. BC has a relatively short lifetime in the atmosphere, in the order of days, which implies that emission reductions of BC rich sources would lead to fast responses in climate impacts. However, since it is emitted along with other particulate and gaseous species that have multiple cooling and warming effects, the net-effect of all relevant species should be taken into account when designing emission mitigation strategies. Recent assessments indicate that a set of measures targeting BC could reduce the projected Arctic climate impacts significantly. The reductions would also bring about co-benefits for public health.

SS08-5 Climate change and the effects on Arctic animals
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The Arctic is one of the regions that the Intergovernmental Panel on Climate Change (IPCC) believes will be most affected by climate change. Some of the expected and already documented alterations are: thinning of the ice sheet, less multi-year ice, increased river discharge into the Arctic Basin, accelerating melting of glaciers, warmer surface temperatures and greater inflow of Atlantic water masses. These changes may potentially alter species distributions, food web structures and carbon cycling, and subsequently trophic-dynamics and transport and uptake of POPs into and within the Arctic. The Arctic environment is also characterised by high seasonality in light intensity, primary production, food availability, lipid concentration in organisms, migration of organisms, and ice cover. These 'naturally occurring' factors influence the availability of POPs and their uptake in the organisms and in food webs. In the international polar year project ‘Contaminants in Polar Regions’ (COPOL) we have been able to study the links between climate and contaminants. The COPOL project has shown that accumulation of POPs in marine organisms varies with year and season. However, the seasonal pattern in accumulation and magnification differs depending on the chemical and group of organisms involved. In all species of zooplankton studied, POP concentrations decrease from May to October. This coincides with decreasing POP concentrations in seawater and increasing lipid stores in the plankton during the same period. Investigation of the accumulation and magnification from zooplankton to fish and birds generally identified July as the month when magnification was greatest, since POP concentrations in the fish species and black-legged kittiwakes were highest in July. The fact that POP concentration in zooplankton was observed to decrease from May to October further underlines the magnitude of increased accumulation through the food web. These results indicate that estimates of the uptake and accumulation of POPs are dependent on the time of sampling (i.e. season). The level of POPs in marine birds varies between years. In kittiwakes (pelagic feeder) the levels of PCBs and DDE were 50% higher in 2008 compared to 2007. In common eiders (benthic feeder) concentrations levels were reduced by 50-60% from 2007 to 2008. This shows that diet can exert a great influence on contaminant load. Concentrations of POPs continue to be found at high levels in top-level predators such as glaucous gulls, ivory gull and great skua. Studies investigating effects show that these species are influenced by the current contaminant loads.

Knowledge about the significance of POPs is essential to be able to differentiate between seasonal variation in accumulation and magnification of POPs versus alterations caused by climate change. Identifying possible alterations caused by climate change in a seasonal environment is a complex and difficult task. Clearly, climate change has the potential to alter POP concentrations in the Arctic. However, the degree to which these concentrations will change, and whether a warmer climate will bring a net increase or decrease of POPs in the Arctic, is still uncertain.

SS09-1 Designing an ecosystem approach for ecological risk assessment of radiation: a path forward for radiocology
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The current approach to ecological risk assessment of radiation based upon “reference organisms” evolved from the radio-toxicological methodology designed to support the development of the system of human radioprotection (concept of “reference man”). Exploiting knowledge on the mechanistic aspects of radiation effects on living matter, it integrates therefore classical ecotoxicological data for some test species along different effect endpoints gathered for individual organisms. As such, it relies exclusively on
the dose-response relationships at the organism level. The approach is limited in light of the extent of both, biodiversity and interaction among species in the environment.

One of the major difficulties in the implementation of an ecological risk assessment is a lack of knowledge about the effects from chronic low-level exposures to radioactive contaminants. To understand the biological role of elements and the trophic level of organisms for transfer of radionuclides to biota.

Importance of the biological role of elements and the trophic level of organisms for transfer of radionuclides to biota.

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3. New requirements in environmental radiation protection imply an increased need for estimations of exposure to biota from radionuclides by risk assessors. A large number of radionuclides that will require assessment in combination with knowledge gaps and complex relations for many radionuclides concerning uptake in biota increases the need to make generalizing assumptions in risk assessments. If an element is biologically essential for an organism this may increase the likelihood of it being taken up, e.g., from food or soil. By that principle, a radioactive isotope of an essential element or a radionuclide with similar characteristics as an essential element might be more likely to be taken up by an organism than other radionuclides. Further, if an organism has in the food web i.e., its trophic level, can affect its uptake of radionuclides. For example, uptake pathways for primary producers and consumers vary greatly. The study explores the importance of the biological role of elements and trophic level of organisms for the transfer of radionuclides. The concentration ranges (CRs) for radionuclides and reference organisms in the ERICA tool (July 2007) that originate from empirical data and the new CRs compiled by IAEA and ICRP in the Wildlife Transfer Database (June 2011) for terrestrial, freshwater, marine and brackish ecosystems were used to study patterns in the transfer of radionuclides to biota. The CRs were divided into three element groups on the basis of their biological requirement (major essential, trace, and non-essential). Further, the reference organisms were divided into groups of trophic level (primary producers, consumers etc.). The results show that CR values are different for the ecosystems. Moreover, the results suggest that for terrestrial biota radionuclides of essential elements and radionuclides with similar characteristics to essential elements are more likely to be taken up than radionuclides of non-essential elements. In aquatic ecosystems some elements have higher CR values than the trophic levels. Recognizing the large knowledge gaps for many radionuclides and organisms concerning transfer to biota this type of grouping of elements and organisms can have many resources in future risk assessments.

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The toxic effect of uranium (U) has not been studied extensively for non-human biota, particularly for aquatic invertebrates. The toxic action of U in organisms potentially originates from both its chemical and radioactive specific activity of U and the interactions with its and associated energy radiation. However, information about distinguishing the hazardous effects of its chemotoxicity and radioactivity on aquatic organisms is scarce. The aim of this study was to identify the contribution of the chemotoxicity and the radionuclides to U on mitochondria and the oxidative balance using transcriptional responses (mt, sod, MnSOD, atp9, cox1, ap6, 12S) and enzymatic activity as endpoints (SOD, CAT, GPx, GST). The opportunity was also taken to evaluate the sensitivity of the used biomarkers by comparing the impacts on the same organ of organisms after a low contamination level. Therefore, groups of organisms (clarks) were exposed for 4 and 10 days to either 30 µg/L of depleted uranium (DU) or 233U which only differ from each other in their specific activity (DU = 1.7 x 10^4 Bq.g^-1, 233U = 3.57 x 10^8 Bq.g^-1) and hence in their radionuclides. In this concentration range is close to some environmental values measured near U mining sites and is twice higher than the world health organization recommendation value for drinking-water. U accumulation levels were measured in different organs (gills, hepatopancreas (HP), stomach, intestine, green gland, muscles, and carapace) whereas biological effects of the different types of U were evaluated only in the gills and the HP, because they provided sufficient amount of tissues for conducting various analyses on the same organ. In order to evaluate the radiotoxicity of both DU and 233U, internal dose rates were calculated with EDEN-2.2 software in the HP for the two sets of experiments. Results showed a significant U accumulation in organs of P. clarkii and some effects on the studied biological parameters (mitochondrial damage and antioxidant response). But despite the huge difference of the specific activities (21000x) between DU and 233U, few significant differences in biological responses were noticed for these two pollutants, indicating that the radiotoxicity was low compared to the chemotoxicity in our experimental conditions. Finally, endpoints measured by genes expression levels gave more sensitive responses than those expressed by enzymatic activities.

SS09-8

Importance of the biological role of elements and the trophic level of organisms for transfer of radionuclides to biota.

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2. New requirements in environmental radiation protection imply an increased need for estimations of exposure to biota from radionuclides by risk assessors. A large number of radionuclides that will require assessment in combination with knowledge gaps and complex relations for many radionuclides concerning uptake in biota increases the need to make generalizing assumptions in risk assessments. If an element is biologically essential for an organism this may increase the likelihood of it being taken up, e.g., from food or soil. By that principle, a radioactive isotope of an essential element or a radionuclide with similar characteristics as an essential element might be more likely to be taken up by an organism than other radionuclides. Further, if an organism has in the food web, i.e., its trophic level, can affect its uptake of radionuclides. For example, uptake pathways for primary producers and consumers vary greatly. The study explores the importance of the biological role of elements and trophic level of organisms for the transfer of radionuclides. The concentration ranges (CRs) for radionuclides and reference organisms in the ERICA tool (July 2007) that originate from empirical data and the new CRs compiled by IAEA and ICRP in the Wildlife Transfer Database (June 2011) for terrestrial, freshwater, marine and brackish ecosystems were used to study patterns in the transfer of radionuclides to biota. The CRs were divided into three element groups on the basis of their biological requirement (major essential, trace, and non-essential). Further, the reference organisms were divided into groups of trophic level (primary producers, consumers etc.). The results show that CR values are different for the ecosystems. Moreover, the results suggest that for terrestrial biota radionuclides of essential elements and radionuclides with similar characteristics as an essential element are more likely to be taken up than radionuclides of non-essential elements. In aquatic ecosystems some elements have higher CR values than the trophic levels. Recognizing the large knowledge gaps for many radionuclides and organisms concerning transfer to biota this type of grouping of elements and organisms can have many resources in future risk assessments.

SS09-9

Effects of chronic radiation exposure on plant populations.

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One of the major difficulties in the implementation of an ecological risk assessment is a lack of knowledge about the effects from chronic low-level exposures to radioactive contaminants. To understand the biological role of elements and the trophic level of organisms for transfer of radionuclides to biota.

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Chernobyl and recent Fukushima Daiichi nuclear disaster revives the importance of understanding the transfer of radioactive contamination in the environment and its ecological consequences. While some studies have been performed on higher organisms, only a few focused on bacterial communities. It is well known that bacteria play an essential role in contaminant mobility in soils by lowering or enhancing their transfer to other compartments (e.g., water, plant, animals). Radionuclides (RN) contaminants...
might also exert toxic effects on bacteria hence inhibiting their role in the transfer. Thus, the objective of this study was to evaluate the impact of RNs contamination on the bacterial community level by its phylogenetic diversity. Following the Chernobyl nuclear accident, contaminated soils, vegetation, and other radioactive debris were buried in situ in trenches. In this area, the trench n°22 has been a pilot site for the study of RNs migration in soil for many years. In a previous study, the bacterial diversity in the area was assessed on a set of control soil samples on this pilot site using a genetic fingerprint method (DGGE). This analysis revealed the presence of complex communities in the soil but did not give access to the taxonomic diversity. To refine the results, an in-depth analysis of the same samples has been conducted by a new high throughput sequencing method, pyrosequencing, leading to 19,000 sequences per sample in average. This molecular technique gave access to unprecedented results and evidenced a huge diversity in the soils with 963 genera and 39 phyla represented. The 4 most predominant phyla, detected in all samples, were Chloroflexi, Proteobacteria, Firmicutes, and Verrucomicrobia. These data demonstrated definitively that a long term exposure to RNs did not lead to the decrease of bacterial diversity as concluded from the DGGE analysis. However, statistical analysis of the pyrosequencing data evidenced a distinction of bacterial community between contaminated and control samples, suggesting the presence of RN adapted species in the contaminated samples. The pyrosequencing data will guide us for the selection of a model bacteria among a collection of 250 cultivable isolates retrieved from these contaminated and non contaminated areas. This model strain will be further used for laboratory experiments to study interactions with representative RNs of the trench (137Cs, U, 90Sr).

Bioaccumulation of Fukushima-derived radionuclides by local marine biota
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SS09-7

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SS09-8

The effect of radionuclide contamination of the Yenisei River on cytogenetic characteristics of aquatic plants
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The Yenisei River, one of the world’s largest rivers, is contaminated with artificial radionuclides released by one of the Russian facilities producing weapons-grade plutonium (the Mining-and-Chemical Combine, MCC), which has been in operation for many years. Aquatic plants are an important component of water ecosystems, which can accumulate high levels of radionuclides and, thus, can be used in biomonitoring and bioremediation. Internal dose rates to aquatic organisms of the Yenisei River were previously estimated for the area near the MCC. The water moss (Fontinalis antipyretica) accumulated the largest artificial exposure dose among the study aquatic organisms. Preliminary results showed that at the MCC discharge site the occurrence of chromosomal aberrations in cells of Elodea was considerably higher than in the control areas. However, plants growing in other parts of the Yenisei, including those with elevated uranium levels, have not been either analyzed for radionuclides or examined cytogenetically. The purpose of the study was to assess levels of radionuclides and to evaluate the frequency of chromosomal aberrations in samples of submerged plants, collected in different parts of the Yenisei river basin, in order to study the following species: Potamogeton lucens, Potamogeton crispus, Potamogeton gramineus, Potamogeton dumerus and various Potamogeton species. Detailed analysis of radioactive contamination of aquatic plants of the Yenisei River revealed large-scale contamination of aquatic plants as far as 230 km downstream of the MCC. About 30 radionuclides, including uranium and transuranium elements, were detected in the biomass of aquatic plants. The highest contamination levels of the major radionuclides for Potamogeton lucens. Results of cytogenetic investigations of aquatic plants suggest that at the MCC discharge site and downstream the occurrence of chromosomal aberrations in ana-telephase and metaphase cells of the plants was considerably higher (up to 30%) than in the control areas (6%). Cytogenetic studies of Elodea canadensis samples collected at positions with elevated uranium levels (and decreased levels of artificial radionuclides) showed that the overall frequency of chromosomal aberrations reached 18%. Thus, not only artificial radionuclides but also uranium concentrated in the biomass of aquatic plants can be responsible for cytogenetic aberrations observed in them.

SS09-9

Speciation, bioavailability and toxicity of uranium in different Lemma minor growth media
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Anthropogenic activities have led to a local increase of U concentration to levels that pose potential ecological risks. Uranium toxicity is known to depend on its redox state, speciation and physiological form. The speciation of U strongly varies with parameters such as pH, dissolved organic matter, carbonate and phosphate concentrations and water hardness. The objective of this work was to investigate the bioavailability and toxicity of U to the freshwater macrophyte Lemna minor L. using the standard growth inhibition test [1]. However, as the bioavailable fraction of U strongly depends on the medium composition the total U concentration, speciation and growth of Lemma in different growth media was evaluated. Three different growth media were selected based on their previous use in Lemma growth inhibition tests: (i) the OECD medium [1], (ii) K-medium [2], and (iii) a synthetic freshwater [3]. For each of the media the pH, CO3 and phosphate concentrations were varied. As expected the OECD medium with normal phosphate concentrations (10.4 mg/L) and the K-medium (29.1 mg/L) enhanced the pH (figure 1A) compared with the OECD (13.8 mg/L) or the K-medium (14.8 mg/L). The pH figure (1A) and the pH of the OECD medium (13.8 mg/L) was similar for all the tested growth parameters. On the other hand lowering the phosphate levels in the OECD medium clearly negatively influenced growth rate. In contrast for the K-medium it was shown that lowering the phosphate concentration did not adversely influence the growth rate (figure 1B). Only when no phosphate was added the growth rate was below 0.250 average growth rate (0.155/2 days). Toward the bioavailability of U only in the K-medium with phosphate concentrations of 0.5mg/L or below U in solution could be retrieved. A dose-response curve for U was set up (0.05mM up to 50mM). In the K-medium with low phosphate concentrations U concentrations from 50mM or higher induced more than 50% growth inhibition in the plants. In contrast in all other tested media similar concentration did not induce growth inhibiting effects probably due to a changed U speciation. The speciation in the different media is currently under investigations [1]. OECD, 2006 Lemma sp. growth inhibition tests. Guideline 221[2] Cederberg, N, et al., 2007 Env. Tox. Chem., 26(1):149-156.[3] Charles, AL, SJ Markich, and P Ralph, 2006 Chemosphere. 62(8):1224-1233.

SS09-10

Change of radioactivity concentration in tree leaves before and after abscission
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Due to the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident, several large releases of radionuclides occurred in March, 2011, and in early April, radionuclide releases had dropped compared to the amounts released in March. National Institute of Radiological Sciences (NIRS) located in Chiba, where 220 km away from FDNPP, also had radioactive deposition from FDNP. In newly emerged tree leaves collected at NIRS in late April 2011, radionuclides were found although they had not contaminated directly from the deposition. According to the previous reports and our observation1-3), we concluded that radionuclide was absorbed from tree surface, e.g. leaves (old leaves of evergreen trees) and stems; radioactivity uptake through roots would be negligible. In an evergreen tree leaves, and Mg, Al, Fe, Cu, Zn, Sr, Cs, etc., were measured. Radioactivity and stable cesium concentrations decreased in dead leaves than that in living leaves for deciduous trees, while evergreen tree leaves tended to show no change before and after fall. Potassium in dead leaves for evergreen tree were less than 0.250. Thus, for most trees, Cs in leaves could be withdrawn to the tree body before fall, and which possibly could be depended on tree types and nutritional condition of the tree. More details will be discussed at the presentation. This work has been partially supported by the Agency for Natural Resources and Energy, the Ministry of Economy, Trade and Industry (METI), Japan.

References
Dose-dependent effects induced by uranium at pH 4.5 in Arabidopsis thaliana

SS09-12

Environmental sensitivity as a tool for the risk assessment of the use of nuclear energy

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Approaches to the management of the risk in radiocology have to take into account geographic, climatic, living and dietary habit differences and in more detail ecosystem differences. The understanding of the factors of sensitivity of different environments, populations or geographic areas is important for scientists and policy makers, to set priorities for the allocation of limited resources. Furthermore the identification of vulnerable environments will be valuable in planning the locations of new nuclear facilities.

A Task Group on Radiological sensitivity was organized by the International Union of Radiocology (IUR) in 2007 on the basis of studies of the Radiocological Sensitivity Forum, 1998-2001. The objective was to discuss a standardization to represent the radiological state of the environment following accidental pollution and a scale of radiocological sensitivity of areas, useful in emergency planning and preparedness. The work of the Group continued under the International Atomic Energy Agency (IAEA) EMA-EMAS II Programme, from 2009 to 2011, as Working Group 8 on Environmental Sensitivity. The WG8 focused its studies on sensitive non-urban environments.

The concept of radiocapacity: theory and application for estimations of ecological risks in ecosystems

Y. Kutlahmedov, I. Matveeva

SS09-11

Agricultural land management options following large-scale environmental contamination

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The accident at the Fukushima Dai-ichi Nuclear Power Plant has raised questions about the accumulation of radionuclides in soil and the transfer to the food chain. Following the Chernobyl nuclear accident, the application of countermeasures is a key issue. Numerous countermeasures were developed since the Chernobyl accident and applied on large scale. This presentation discusses countermeasure strategies and their effectiveness and feasibility against the background of the Fukushima Dai-ichi nuclear accident and the agricultural areas affected. Land management options should be selected based on a criteria such as effectiveness; constraints on implementation; wastes generated and waste management options; doses received during implementation; side-effects; cost/benefit considerations; acceptance (stakeholder opinion).Mechanical (soil removal, ploughing) and agrochemical (e.g. application of fertilizers or caesium sorbents) management options will be discussed in terms of their potential effectiveness in reducing the soil-plant transfer but also potential side effects will be highlighted. Specific attention will be attributed to andosols, a prevailing soil group in Japan. In addition phytomass agromant options such as food crop collection and cultivation of technical crops (e.g. bioenergy crops, fibre crops) will be briefly discussed. Phytoextraction will be critically examined. Land management options will be evaluated based on available information on soil contamination levels, soil characteristics, food and non-food crop production and conversion systems in Japan and following interaction with institutes belonging to NARO (Japanese National Agricultural and food Research Organisation), and considering the evaluation by international teams of the remediation activities in Japan. For optimizing agricultural management options, a good knowledge on agricultural practice and soil characteristics is required. Alternative land uses for areas were contamination levels are considered too high, need careful evaluation. Planning for agricultural management options requires a holistic approach, considering radiocological, radiological, environmental, economic and socio-cultural and political aspects.

SS09-10

Dose-dependent effects induced by uranium at pH 4.5 in Arabidopsis thaliana

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U-content in roots and shoots increased with increasing U-concentration added to the nutrient solution. However, there was a low root-to-shoot transfer. Fresh weight of roots and leaves decreased after exposure to 50, 75 and 100 µM U. In contrast, plants exposed to 6.25 and 12.5 µM U had an increased fresh weight as compared to the control plants, which alules to a hormesis effect as was observed before (Vanhoudt et al., 2008, Stracevz et al., 2009).One of the most important physiological processes in plants is photosynthesis. Apparently, the photosynthetic efficiency of plants exposed to 25-100 µM U increased. This indicates that the photosynthetic system is not damaged but in contrast plants try to optimize their photosynthesis under U-stress. In addition to the reduced biomass production, plants exposed to 25-100 µM U showed an increased lipid peroxidation. This increase indicates an affected membrane integrity and functionality. Assessing antioxidant enzyme capacity of the plants indicated an increased defence against ROS in the U-exposed plants as evidenced by increased activities of ROS scavenging enzymes. The increased activity of guaiacol peroxidase could indicate an increased cell wall lignification as a defense reaction that limits the entry of toxic metals. In conclusion, this study indicates that elevated U-concentrations at low pH can cause important morphological, physiological and biochemical effects in Arabidopsis thaliana seedlings.

SS09-13

Radiocesium distribution in a bamboo forest

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The concept of radiocapacity: theory and application for estimations of ecological risks in ecosystems

Y. Kutlahmedov, I. Matveeva

SS09-1

Bioavailability and bioavailability of radionuclides in ecosystems

K. Krylov, S. Kukharenko, A. Fedoseyev

The transfer in the food chain. Follow-

ing the Fukushima Dai-ichi Nuclear Power Plant has raised questions about the accumulation of radionuclides in soils and the transfer to the food chain. Following the Chernobyl nuclear accident, the application of countermeasures is a key issue. Numerous countermeasures were developed since the Chernobyl accident and applied on large scale. This presentation discusses countermeasure strategies and their effectiveness and feasibility against the background of the Fukushima Dai-ichi nuclear accident and the agricultural areas affected. Land management options should be selected based on a criteria such as effectiveness; constraints on implementation; wastes generated and waste management options; doses received during implementation; side-effects; cost/benefit considerations; acceptance (stakeholder opinion).Mechanical (soil removal, ploughing) and agrochemical (e.g. application of fertilizers or caesium sorbents) management options will be discussed in terms of their potential effectiveness in reducing the soil-plant transfer but also potential side effects will be highlighted. Specific attention will be attributed to andosols, a prevailing soil group in Japan. In addition phytomass agromant options such as food crop collection and cultivation of technical crops (e.g. bioenergy crops, fibre crops) will be briefly discussed. Phytoextraction will be critically examined. Land management options will be evaluated based on available information on soil contamination levels, soil characteristics, food and non-food crop production and conversion systems in Japan and following interaction with institutes belonging to NARO (Japanese National Agricultural and food Research Organisation), and considering the evaluation by international teams of the remediation activities in Japan. For optimizing agricultural management options, a good knowledge on agricultural practice and soil characteristics is required. Alternative land uses for areas were contamination levels are considered too high, need careful evaluation. Planning for agricultural management options requires a holistic approach, considering radiocological, radiological, environmental, economic and socio-cultural and political aspects.

SS09-2

Radiocesium distribution in a bamboo forest

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Bioavailability and bioavailability of radionuclides in ecosystems

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The concept of radiocapacity: theory and application for estimations of ecological risks in ecosystems

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After the accident in Chernobyl, the most part of Ukraine has been contaminated with cesium-137. Meanwhile, cesium is the closest analogue of potassium that is known as the macro element of great importance. Therefore, 137Cs has turned out to become the inescapable tracer in biological objects of all ecosystems in Ukraine, almost without exceptions. Thus, there is every reason to believe that the monitoring of this radionuclide can be employed for quantitative estimations of robustness of biota in the ecosystems. We suggest the concept of radio-capacity, as the novel approach, for estimation and prognostication of the states of ecosystems biota exposed to unfavorable physical and chemical factors. The radio-capacity is defined as the threshold (limit) amount of 137Cs, i.e. - the amount at which there are still no visible violations in the basic functions of biota, such as the capability to retain the biota biomass and the environmental conditions. Besides, basing on this concept and the theory of reliability, we have developed the mathematical (stochastic) model of the radio-capacity. Comparison analysis of the data for different kinds of ecosystems, including terrestrial, aquatic, forest, grasslands and urban landscapes, demonstrates that the parameters of our reliability-theory model are adequately sensitive to the impacts of irradiation and heavy metals on the biota. We show that both, distribution and redistribution, of the cesium-tracer in aquatic and terrestrial ecosystems nearly follow all essential external factors, like the changes in climate, floods, contra-measures, and so on, as well as they respond to different kinds of contaminations, like the ionizing radiation, heat fluxes from atomic power stations and chemical pollutants. The higher is the radio-capacity factor, the greater is the biota robustness and the less is the ecological risk for the biota. Moreover, we have demonstrated the key role of the preparation and storage processes of exposure of the biota simultaneously to radiation and chemical pollutants. Thervewith, the interference of the different factors may change from synergism to antagonism. Thus, it may be concluded that the radio-capacity holds much more promise as the unified quantitative parameter for quantitative estimations of impacts of different factors on the ecosystems biota. In addition, basing on the radio-capacity concept, we suggest the method of ecological standardization that is prospective to determine the threshold levels of pollutants acceptable for the ecosystems biota, as well as to assess the levels of ecological risks.
In October, we collected samples from the same forest again. The Cs-137 concentrations in the leaves decreased by ca. 100-fold, and that in the litter layer was ca. 6-fold.

nuclide releases from FDNPP.

We collected stems, leaves, shoots, litter, and soil samples of a bamboo forest (Ibaraki Prefecture) on 8 May, 2012 to see the distribution about 2 months after the large radio-

Japanese nuclear forest

The ingestion of radionuclides during food consumption can represent major source of radiation exposure to humans. Uranium, thorium and their decay products, are naturally present in the environment and can be transferred to animals and animal products. Even if the background levels in animal products don’t represent a significant risk due to the low activities of radionuclides, their evaluation is important. Indeed, the assessment of the magnitude of a contamination due to accidental or chronic releases of radionuclides depend on the knowledge of the natural activities. The present study aims in the first instance to quantify the activities of uranium, thorium and their decay products in chicken meat and eggs in five regions of the French territory. The highest activities (mBq/kg; 1 fresh weight) are measured for radium isotopes in eggs and range between 136 and 190 for 226Ra. In comparison, uranium activities in eggs are much lower and range between 0.51 and 1.30 for 238U. In chicken meat, 238U activity concentrations show higher values than in eggs and range between 1.7 and 9.7. Concerning 232Th, its activities are lower than those of 238U and range between 0.5 and 4.9. Secondly the contribution of the potential sources of the radionuclide for chicken meat and eggs (grain mixture, soil particles and drinking water) are studied in order to calculate the concentration ratios and the transfer factors. These values could enrich the data given by the technical report series N472 of IAEA (2010). In this report the mean transfer factors for uranium in chicken meat and eggs (0.75 d.kg⁻¹ and 1.1 d.kg⁻¹ respectively) are only based on two results from the study of Prister (1967). Moreover no concentration ratios are given up to now in the literature for uranium, radium and thorium in eggs and chicken meat. It is important to complete this database, to provide reference transfer coefficient values which could be used to predict the behaviours of radionuclides in the environment.

Annual intakes of ²²⁴Ra,²²⁶Ra and ²³⁸U in staple foodstuffs from a high background radiation area in the southwest region of Cameroon

Ele Abiama P., Ben-Bolie G.H., Amechmachi N., Najib F., El Khoukhi T., Owono Ateba P.

Annual intakes of 226Ra, 228Ra and 40K in staple foodstuffs from a high background radiation area in the southwest region of Cameroon

Complementary to the collected radionuclides, 226Ra, 228Ra and 40K were determined in five most consumed vegetables in a high-level background radiation area (HLBRA) in the southwest region of Cameroon. A total of 25 foodstuff samples collected from Akongo, Ngombas, Awanda, Bikoué and Lolodorf rural districts were analyzed by gamma spectrometry. The average activity concentration values of 226Ra, 228Ra and 40K were respectively 2.30, 1.50 and 140.40 Bq·kg⁻¹ fresh-weights. The effective dose for individual consumption of the investigated foodstuff types was calculated on an estimated annual intake of such diets for Adults, Children and Infants in the study area. The total effective doses from the daily intake of the investigated foodstuffs for each studied long-life natural radionuclide were respectively 0.41μSv for 226Ra, 0.84μSv for 228Ra and 0.71μSv for 40K. The total annual effective dose was estimated at 0.70 mSv y⁻¹. 228Ra (44%) and 40K (36%) were found to be the main sources for internal irradiation which is very likely due to the specific uptake of these radionuclides by the studied plants.

Incorporating plant physiological pathways to mechanistic modelling soil-to-plant transfer of radionuclides

T. Sauras, J. Casadesus, R. Vallejo

BIORUR is a mechanistic model developed to assess the soil-to-plant transfer of radionuclides. BIORUR algorithms assume that the movement of radionuclides in an ecosystem and inside the plants is through the same network of pathways than nutrient cycling. The fluxes of radionuclides between compartments inside an ecosystem or a plant are estimated from the flux of a nutrient analogue between these compartments, the ratio of concentration radionuclide to nutrient and a selectivity coefficient which compares the affinity of the pathway for radionuclide to that for its nutrient analogue. One advantage of BIORUR algorithms is the flexibility to adapt the model to a wide range of scenarios where the only requirements are that nutrient cycling have been quantified and that each radionuclide must be associated to a nutrient analogue. However, the scope of radionuclides is restricted to those which are associated to a nutrient analogue, such as Cs-K, Sr-Ca. BIORUR model incorporates some of the biological processes relevant to radionuclide availability such as plant physiology, mycorrhizal transfer, organic matter mineralisation and micro-organism. In order to study the plant physiological mechanisms involved, we have developed a hydroponics plant growing system in which we manipulate the plant-environmental processes affecting the uptake of radionuclides (i.e. transpiration, plant growth rate, nutrient demand, nutrient supply) . The experimental system permits to verify the mechanisms of Cs and Sr uptake already included in BIORUR.

REACH: what can be learned from the first registration deadline?

W. De Wolf

European Chemicals Agency - ECHA, Helsinki, Finland

The first REACH registration deadline for substances produced at 1000 tonnes or more per year, carcinogenic/mutagenic/reprotoxic substances (CMRs) and substances of very high concern (SVHC) was in 31 May 2013. CAU, on behalf of the UK and in partnership with the Netherlands and Germany, led on this work. The revisions mean that fewer animals can be used in the existing aqueous test in certain cases and, for highly hydrophobic substances, a dietary exposure test has been added. The revised test guideline has more detail for interpreting test results, as our understanding of important factors in laboratory aquatic bioaccumulation testing has grown. The major changes concerning data treatment and interpretation relevant to B. CAU can be used in all the EU Annexes XIII of REACH, which lists the PBT/vPvB criteria, has been revised introducing additional ways to capture substances as being 'PBT'-like, or of equivalent concern to PBT substances, even though they do not meet the numerical criteria of degradation half-life, aquatic bioconcentration factor and long-range transport. Some of these additions to the annex will be discussed and illustrated with practical examples of these changes that CAU have been working on.

The OECD Test Guideline (TG) for measuring aquatic bioconcentration (TG 305), a very important guideline for both risk assessment and PBT assessment, has been a subject of the revision last the four last years. CAU, on behalf of the UK and in partnership with the Netherlands and Germany, led on this work. The revisions mean that fewer animals can be used in the existing aqueous test in certain cases and, for highly hydrophobic substances, a dietary exposure test has been added. The revised test guideline has more detail for interpreting test results, as our understanding of important factors in laboratory aquatic bioaccumulation testing has grown. The major changes concerning data treatment and interpretation relevant to B. CAU can be used in all the EU Annexes XIII of REACH, which lists the PBT/vPvB criteria, has been revised introducing additional ways to capture substances as being 'PBT'-like, or of equivalent concern to PBT substances, even though they do not meet the numerical criteria of degradation half-life, aquatic bioconcentration factor and long-range transport. Some of these additions to the annex will be discussed and illustrated with practical examples of these changes that CAU have been working on.

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Updates of dossiers in light of new scientific data: expectations on what is regarded as new in a practical way. What are the problems and challenges for the future?

E. Douben
REACHwise, Nederland

REACH places a duty on registrants to keep their dossiers up to date. Amongst those possible update reasons is new scientific data. This begs the question what the boundaries of this concept are, and what it means in a practical way. Does the discovery in a lab trigger updating obligations or not? In other words what are possible criteria, time line, etc. The issue cannot be seen in isolation but must be placed in the entire framework of operations in which companies operate to meet their legal obligations.

What can be learned from the first registration deadline? Expectations for the next registration deadline

N. Nimpuno
ChemSec, GOTEBOGS, Sweden

NGOs had very high expectations on the first registration deadline. At last, large amounts of information on individual substances would be published by ECHA, giving the general public, authorities, scientists, as well as NGOs, access to high quality environmental information for all high tonnage substances. However, the reality has instead caused concern due to delays in assessing dossier information. This makes it impossible for non-authorities to connect the submitted information to a responsible company. Further, many registration dossiers have proven to be of poor quality, and there are data gaps for key parameters from higher-tier studies such as endocrine disrupting properties, persistence and environmental fate. The lack of correct and sufficient data in the registration dossier can adversely affect enforcement, with enforcing activities already strained. For the coming registration deadline in 2013, an emphasis must therefore be placed on correctly compiled registration dossiers from companies and a rapid dissemination of the submitted information by ECHA. NGOs call for the dissemination portal to flag the status of assessing confidentiality claims on data that has been claimed as confidential by the registrants. NGOs also call for other information such as the source of the submission of substances already in use in the EU, as for many there was inadequate information on their hazards and risks they pose.

REACH registrants have to provide information on the intrinsic properties and hazards of the substance in the registration dossier. The standard information required depends on the tonnage manufactured or imported; the higher the tonnage, the more information needed. In addition for substances at 10 tonnes per annum (tpa) or above, the registration dossier must include a Chemical Safety Assessment of chemical substances

The overall purpose of both the REACH (EC No 1907/2006) and the CLP (EC No 1272/2008) Regulations is to ensure a high level of protection of human health and the environment. Industry has to ensure that chemical substances are used safely. A key motivation for developing REACH was to fill information gaps for the large number of substances already in use in the EU, as for many there was inadequate information on their hazards and risks they pose. REACH registrants have to provide information on the intrinsic properties and hazards of the substance in the registration dossier. The standard information required depends on the tonnage manufactured or imported; the higher the tonnage, the more information needed. In addition for substances at 10 tonnes per annum (tpa) or above, the registration dossier must include a Chemical Safety Assessment of chemical substances

The purpose of Chemical Substances Control Law (CSCL) is to evaluate, before manufacture or import, whether or not new chemical substances have properties such as persistence, and to implement necessary regulations, in order to prevent environmental pollution caused by chemical substances that pose a risk of impairing human health or interfering with the inhabitation and/or growth of flora and fauna.

To minimize the significant adverse effect of chemical substances on human health and environment by 2020 (Agreement in the Environmental Summit in 2002), CSCL has been amended in 2009 and implemented in 2011. Under amended CSCL, risk assessment will be conducted in step-wise manner for all chemicals including existing chemicals. In this presentation, chemical risk assessment approach under amended CSCL and the results of comparison between CSCL and REACH will be introduced.
The reviewed in-vitro approaches are promising tools for identification and prioritization of chemicals in bioaccumulation assessment. Their application in regulatory assessment can be estimated using a variety of in-vitro systems including hepatocytes and microsomal or S9 fractions of fish liver in combination with a physiologically based prediction model. A permeability assay that is tuned to hydrophobic chemicals, for which resistance of overall permeability is aqueous boundary layer controlled. In-vivo metabolism rate can be explored as potential indicators for chronic toxicity, bioaccumulation, and endocrine disruption determinations. Ample room exists for improved use of in vitro, embryo tests, threshold approaches, and implementation of robust experimental designs. The overall goal is to minimize animal use while maximizing the collection of information useful in hazard and risk assessment, and classification and labelling. Method development needs and future opportunities will be identified throughout.

The validation journey - What is validation and how does it work?

K. Schirmer
Eawag, DUBENDORF, Switzerland

Fish cell-based systems hold great potential for deciphering the molecular mode of action of chemicals and, provided the right choice of in vitro model and exposure conditions, may supplement or even substitute fish toxicity tests. Conceptually, if the cell-based responses reflect the initial stage for an adverse outcome seen at the organism level, it must be possible to develop an array of cellular systems suitable to identify mechanisms of action and adverse outcomes. In support of this concept, we have recently demonstrated that a gill cell line from rainbow trout (Oncorynchus mykiss), RTgill-W1, responds with a loss of cell viability at comparable effect concentrations as fish responding with death in acute exposure scenarios for a wide range of organic industrial chemicals with different modes of actions. The gill epithelium is the primary uptake site of water-born contaminants into fish; thus, damage to gill epithelia can be seen as the link to an impairment of the whole organism and eventually death. Transferring this concept to adverse outcomes other than acute lethality certainly requires more sophistication. For example, cell-based models could be used to elucidate the role of epithelial barriers (such as gills, intestine) or tissues (such as liver) in transforming and excreting chemicals. Different cell cultures may be put together, e.g. on microfluidic devices, to better resemble the interaction of different cell types and transport of bioaccumulation products and signaling molecules from one compartment to another. Moreover, molecular mechanisms of toxicant action can be identified as part of a tiered testing approach but as well, cellular parameters, such as ATP production or proliferation should be explored as potential indicators for, e.g. reduced growth in fish. If these systems are combined in an intelligent way and systematically linked to chemical concentrations and response pathways and outcomes in fish via computational approaches, they provide a foundation for the development of a “virtual fish”.

In-vitro methods for bioaccumulation: a modular approach including passive uptake and metabolism assessment

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Predictive tools for the assessment of bioaccumulation are critical components in an overall strategy for assessing bioaccumulation potential. This presentation reviews the potential of in-vitro test systems for improved prioritization and assessment of potentially bioaccumulative chemicals. The toxicokinetic processes of adsorption, distribution, metabolism, and elimination (ADME) determine the bioaccumulation of hydrophobic organic compounds in fish and mammals. Mechanistic models of bioaccumulation explicitly consider these ADME processes but there is a lack of appropriate data as model input parameters, particularly for compounds that do not partition simply to tissue lipid. There are a variety of in-vitro systems available for estimating ADME properties. Here we explore the applicability of these in-vitro assays for estimating ADME input parameters for bioaccumulation models. Caco2 cell lines, the parallel artificial membrane permeability assay and related assays can only be applied for rather hydrophilic chemicals and need adaptation for hydrophobic chemicals. Passive absorption and elimination through fish gills can be described by a modified parallel artificial membrane permeability assay that is tuned to hydrophobic chemicals, for which resistance of overall permeability is aqueous boundary layer controlled. In-vitro metabolism rate can be estimated using a variety of in-vitro systems including hepatocytes and microsomal or S9 fractions of fish liver in combination with a physiologically based prediction model. The reviewed in-vitro approaches are promising tools for identification and prioritization of chemicals in bioaccumulation assessment. Their application in regulatory assessment schemes is relatively new and will require continued validation to fully elucidate their potential advantages and limitations.

The EUROECOTOX EU-7th-framework project - identification of gaps and limiting steps for reduction, replacement and/or refinement of animal experiments used in environmental risk assessment

5 Scholes, I, Blaha1, T Brauneck1, M Galay-Burgos1, M Garcia-Franco2, J Guineau1, N Klüver1, K Schirmer1, K Tanneberger1, M Tobor-Kaplon1, H Witters2, E Sela3
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4ECETOX, BRUSSELS, Belgium
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7NOTOX, ‘S HERTOGENBOSCH, The Netherlands
8VITO, MOL, Belgium

Animal experiments play an integral role in current environmental risk assessment for the registration of chemicals, pesticides, biocides, pharmaceuticals and testing of workplace effluents. As with risk assessment in human toxicology, there is a strong societal demand to replace, reduce or refine the animal experiments performed in order to protect the environment. However, efforts to develop and validate alternatives for human risk assessment are relatively more advanced: international OECD guidelines based on alternatives are already available for some endpoints (e.g. skin corrosion, eye irritation, phototoxicity, genotoxicity). EUROECOTOX (European Network for Alternative Testing Strategies in Ecotoxicology) is an European Union coordinated action project which aims at identifying the gaps and limiting steps for reduction, replacement and/or refinement of animal experiments used in environmental risk assessment. EUROECOTOX is performing an analysis of regulatory requirements for ecotoxicity testing, novel
strategies and approaches to reduce vertebrate testing (fish, birds, amphibians), potential bottlenecks for validating new methods and measures to accelerate development and validation of alternatives. Recently, EUROECOTOX has identified the lack of OECD guidelines, the need to consider criteria of validation (reliability and relevance) early in the development, (financial) support for validation of new approaches and limited access to existing animal test data for the scientific community as one of the major limiting issues. The detailed analyses and statistics of currently available alternative approaches and limitations will be given, supplemented by experimental findings from inside the consortium and published studies. The audience is invited to subscribe to the EUROECOTOX network and to discuss measures for advancing the development and acceptance of alternative methods in environmental risk assessment (http://www.euroecotox.eu).

SS12-1
Introduction and resume of approaches to establish critical loads and related concepts for selected environmental impact categories and their applicability to a global chemical footprint

TV Rydberg
JVL and Environmental Research Institute, GOTEBORG, Sweden

The presentation will give an introduction to how the critical load concept, and related concepts to characterise allowable pollution loads, have been developed, and what considerations have been necessary to arrive at agreed levels. The applicability of such concepts to global chemical footprint will be briefly discussed.

SS12-10
Tackling action on high-concern chemicals - The NGO perspective

N Nimpunen
ChemSec, GÖTEBORG, Sweden

The increasing-use of chemicals in virtually every product used in modern life has enabled improvements in society globally. However, the widespread use of chemicals has introduced actual and potential problems to man and the environment on a scale that is virtually impossible to estimate. The rise of different forms of cancer, decrease in reproductive success, increased obesity, emergence of behavioral disorders among children etc. are only some examples of the problems increasingly associated with exposure to high concern chemicals. In parallel with improved tools to measure these effects, and reaching agreement on how to classify the chemicals causing these effects, one must, from an environmental health perspective focus on what to do to prevent damage from occurring in the first place. Our focus is not so much the cut-off values that can potentially be reached before irreversible damage is achieved. Rather, the question also has to be asked: What negative effect of chemicals exposure is accepted? On an individual as well as societal level. Not only how far can we go? But how far are we, as a society willing to go?

SS12-2
Planetary boundaries and chemical pollution

A. De Wit
Stockholm University, STOCKHOLM, Sweden

The concept of planetary boundaries is defined to indicate possible serious anthropogenic disturbances of the function of the Earth system, and chemical pollution might be an important contributor to the overall burden of humans on the planet. The concept as such (Rockstrom, Steffen et al. 2009) want to define a "safe operating space for humanity" in order to maintain the planetary conditions within ranges that we know are safe. The primary example is global warming where several studies point to the possibility of defining a range of radiative forcing within which climate will still be without unexpected serious changes related to nonlinear responses of the system. Is this approach applicable to all forms of chemical pollution? Chemicals may impact through specific mechanisms at sensitive points of the earth system, such as e.g. CFCs on the ozone layer, or due to other effects, more or less specific, on biota. In that case, also possible combined effects on ecosystems of persistent organic pollutants (POPs), endocrine disruptors, plastics, heavy metals and nuclear waste. Due to the large number of chemicals in commerce, it is impossible to measure all possible chemicals in the environment. Another major stumbling block is lack of understanding of the effects of chemical mixtures on organisms, ecosystems and Earth system functions. Two complementary approaches were identified as possible ways to define a planetary boundary for chemical pollution: 1) focus on POPs with global distributions and 2) identify unacceptable, long-term, toxic impacts of chemical pollution on human organs (1). Data for only a few chemicals with POPs characteristics are available, for example DDT, DDE and PCBs. In the second case, boundaries focusing on effects could be based on impacts on reproduction, immune systems and neurobehavior, particularly in sensitive species at sensitive life stages. However, it is currently difficult to link many chemicals to specific effects due to lack of toxicity data. One example of how this approach might work was the observed increase in neurodevelopmental disorders (autism, ADHD etc.) seen in children. A large number of chemicals are known to be neurotoxic in experimental animal and in humans, and five (methyl mercury, arsenic, lead, PCBs, toluene) are known to be toxic to human neurodevelopment. Thus, widespread exposure to low concentrations of many chemicals with known or suspected neurotoxic effects may have created a silent pandemic of neurodevelopmental disorders in children on a global scale (2). [1] Rockström J et al. 2009. A safe operating space for humanity. Nature 461:472-475. [2] Grandjean P, Landrigan PJ. 2006. Developmental neurotoxicity of industrial chemicals. Lancet 368:2167-2178.

SS12-3
Emissions of chemicals - important or misleading for a planetary boundary of chemical pollution?

M Molander
Chalmers University of Technology, GOTHENBURG, Sweden

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SS12-4
Do we have the concepts and tools to estimate the global exposure to anthropogenic chemicals?

M. Scheringer
ETH Zurich, Zürich, Switzerland

Chemical pollution is not only a combination of many local or regional cases of environmental contamination, but has also truly global aspects. These include that there are chemicals that will be distributed around the globe, in particular Persistent Organic Pollutants (POPs), and that there is a global trade in chemicals and chemical wastes that leads to widespread distribution even of chemicals without POP properties. This global nature of the chemical pollution problem is illustrated by the fact that the new UNEP Global Environmental Outlook (GEO-5) [1] will, for the first time, include a chapter on chemicals and wastes. The Planetary Boundaries concept is important because it makes it possible to explicitly address these global aspects of chemical pollution. As an essential element of an assessment of chemical pollution at the global level, we need to quantify the total global emissions to a wide range of chemicals and the resulting global exposure to these chemicals. In this talk, it will be demonstrated that the combination of educated guesses of emission rates and multi-media mass balance models provides a means to reliably estimate the global exposure to anthropogenic chemicals. Global exposure includes levels of chemicals in various environmental media such as air, water, soil, and vegetation and in various regions of the globe, reaching from urban to pristine. For several types of chemicals, model results based on estimates of global emissions show good agreement with field data. It is concluded that today the tools that are needed to assess global exposure to anthropogenic chemicals are available. Because of large data gaps in emissions and chemical property data, these estimates of global exposure to chemicals are still surrounded by uncertainties, but nevertheless a relatively clear picture of the global burden of anthropogenic chemicals can be established. Even on the global scale with maximum dilution by large volumes of water and air, average concentrations in ocean water in the pg/L range are observed for single POP-type chemicals when current emission rates are used. The combination of all relevant chemicals in commerce makes the total concentration of chemicals available for uptake by humans and wildlife even higher. [1] Global Environmental Outlook, to be published by the United Nations Environment Programme in May 2012. http://www.unep.org/geo/

SS12-5
Planetary boundaries: a suitable concept for chemical pollution?

T. Backhaus, M Gustavsson, A. Alvarsson
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The concept of planetary boundaries has been introduced by Rockström et al. in a publication in Nature in 2009. They are defined as 'scientifically informed values of the control variable established by societies at a 'safe' distance from dangerous thresholds'. That is, planetary boundaries indicate critical thresholds that must not be exceeded, in order to avoid catastrophic environmental impacts. Rockström and his colleagues suggested specific planetary boundaries for climate change, ocean acidification, stratospheric...
ozone depletion, land and freshwater use, biodiversity loss and interferences with phosphorus and nitrogen cycling. This has also not yet been quantified. This presentation will therefore explore whether it is indeed possible and sensible to apply the concept of planetary boundaries to toxic chemicals in the environment. Limitations of and challenges for the planetary boundary concept are not only related to emission, fate and exposure assessment (which were discussed in the previous talks). To a large extent they are also connected to the ecotoxicology of chemicals and include (a) the need to account for the ecological effects of complex multi-component mixtures, (b) the ignorance of local conditions and local effects, and (c) the enormous differences in the ecotoxicological profiles of the myriad of chemicals used in society, which, together with the ever-changing chemical use patterns, make any boundary a constantly moving target. We will explore those issues and their consequences for the concept of planetary boundaries for chemical pollution. To this aim we will use two groups of environmentally important chemical groups as cases in point: (1) a particular group of pesticides, so-called photosystem-II inhibiting herbicides, (2) unspecifically acting industrial chemicals (so-called baseline toxicants).

SS12-6
Fate-based categorization of chemicals and GIS Fate Model for the planetary boundaries concept
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It may be difficult to set up single criteria for the Planetary Boundary of chemical pollution, because chemicals have different mechanisms of effects on organisms including humans and different modes of emissions to the environment. First of all, it is necessary to consider simultaneously both the categorization of chemicals based on characteristics and the prioritization of the categorized groups. Categorization of chemicals might be decided as matrix of their characteristics such as environmental fate, toxicities, and other relevant information. As for environmental fate, though POPs (Persistent Organic Pollutants) and POPs-like chemicals should be important, chemicals which are not POPs-like but having temporal/spatial local peak should also be considered in terms of the Planetary Boundaries discussion. For example, if some chemicals locally affect some microorganisms on their reproduction and this impact affect other plants or animals, this issue is no longer a local problem. This means we should consider not only environmental fate of chemicals but also these ultimate impact to the planet. Data aggregation of all available information is also crucial when we select an appropriate method of the chemical risk assessment. Each category of chemicals should have each appropriate method. Although the simplified models like SimpleBox, CaTox, and ChemRange are useful for wide range of chemicals in faster and with less resource, the models are also indispensable that aims to reproduce the chemical fate processes in more details. In this presentation, we introduce our research about the detailed multimedia fate models developed for pesticides (PeCHREM/G-CIEMS) and the global model developed for POPs (FATE). As for the PeCHREM/G-CIEMS, in order to accurately predict the temporal/spatial variations of many pesticides in the environment, we constructed the prediction methods using general statistics of pesticide use, geographical, meteorological, hydrological database. As for the FATE, two issues were important that are to solve the temporally/spatially resolved emission inventory and to solve the general circulation of the atmosphere and the ocean. We also show the possible way to contribute these models to the Planetary Boundaries concept.

SS12-7
A planetary boundary for chemical pollution
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Johan Rockström and co-authors (2009) wrote a persuasive argument that human activity is exceeding the planet's biophysical boundaries. Rockström et al. defined 10 boundaries that we need to live within, or alternatively a radically different future. Chemical pollution was one of the 10 boundaries discussed, but the authors were unable to define the actual boundary. Unlike the other 9 boundaries, defining a boundary for chemical pollution will be challenging because the boundary for chemical pollution does not have a single variable, metric or indicator. We argue that a planetary boundary for chemical pollution is necessary to address the local to global nature of chemical release and transport, and the myriad and often subtle adverse effects that are arising from single and multiple chemical exposures. Furthermore, populations are increasingly vulnerable to adverse effects from chemical pollution as a result of experiencing multiple stresses, i.e., the global system abutting against other planetary boundaries. We suggest starting the planetary boundary discussion by exploring the similarity with the "critical load" concept. A critical load is defined as the highest load that can be added to a system without causing a specified adverse effect in a sensitive population or ecosystem. A critical load is estimated by first choosing a sensitive toxicity endpoint in a sensitive organism, population or ecosystem, from which the corresponding emission rate is back-calculated. For the planetary boundary, the calculation should be done on a spatially resolved global scale with attention paid to ecologically vulnerable systems. Into the calculation we need to specify chemical emissions as tied to annual chemical production and use. Environmental fate processes in the chemical production phase, as well as chemical persistence and bioaccumulation, must also be addressed. Perhaps most challenging, we need to consider multiple chemical emissions rather than take a chemical-by-chemical approach. As a first step, we suggest calculating single-chemical critical loads or planetary boundaries for the few, relatively well understood high production volume chemicals for which we can gather sufficient information on physical-chemical properties, emission rates, inventory, environmental fate, exposure and toxicity. Considering lower production volume chemicals may be unnecessary.

SS12-8
Assessing the principle of a planetary boundary for chemicals used in personal care products
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As the per capita GDP of the world’s population continues to grow, particularly in developing countries, such as Brazil, Russia, India, and China, which are expected to be the four biggest economies by 2050, it is likely that the use of chemicals in commerce that is associated with modern societies will significantly increase over the next several decades. It is thus important that governance approaches are sufficient to inform risk management discussions for reducing environmental impacts related to economic growth, and are based on rigorous scientific assessment of risks. Johan Rockström and colleagues (2009) proposed nine planetary boundaries of safe operating space for humanity. The authors suggest that these boundaries might stimulate a discussion for novel and adaptive governance approaches at the global, regional, and local levels. Included in the nine planetary boundaries is a boundary proposed for chemical pollution, which Rockström and colleagues do not quantitatively define. A number of questions thus follow from the proposal to define a safe operating space for humanity with respect to quantifying a planetary boundary for chemical pollution. For instance, what is meant by safe operating space and what is the relationship with other concepts, such as current approaches to environmental risk assessment, life-cycle assessment, ecosystem services or source to outcome pathways? Is an effort to ensure that the use of chemicals in society associated with economic growth do not negatively impact the environment, we consider here the opportunities that the principle of a planetary boundary for chemical pollution offers, by exploring the utility of existing science-based assessment methods to inform decision making, assessing the role of technological innovation in relation to economic growth as well as issues of spatial and temporal scales, and how these parameters influence our ability to quantify a planetary boundary for chemical pollution.

SS12-9
Sound Chemicals Management - The policy perspective
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The aim of the Strategic Approach to International Chemicals Management is to achieve, by 2020, that throughout their life cycle chemicals are used and produced in ways that lead to the minimalization of significant adverse effects on human health and the environment. While significant progress has been made in some parts of the world, progress is sometimes uneven and that not enough is being done to reach the 2020 WSSD chemicals goal. The unsound management of chemicals can lead to the contamination of air, water and soil, resulting in increased human exposure and associated health risks. Sound chemicals management through the life cycle should be one of the key factors underpinning efforts to achieve poverty eradication and a green economy. The intention of this paper is to give state of art and to draw the attention to some areas where further progress needs to be made.
EC01A-1
PAH, PCB and OCP concentrations in the sediments, local mussels, transplanted mussels and passive samplers in the Istanbul Strait and Marmara Sea, Turkey
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Lipophilic organic pollutants such as polyaromatic hydrocarbons (PAHs) and persistent organic pollutants (POPs) are accumulated in sediments and in organisms. The accumulation of pollutants especially in fish and mussels may lead to serious human health hazards. In this study, the surface sediments and mussels collected from five sites of the Istanbul Strait and Marmara Sea as well as the transplanted mussels and deployed passive samplers were analyzed by using a high resolution mass spectrometer coupled with an gas chromatography to determine the concentrations of PAHs, polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCP). Istanbul Strait is a part of the Turkish Strait system connecting the Black Sea to the Mediterranean and one of the busiest waterways in the world. Maximum sediment T-PAH concentration (6431 ng/g) was measured at the sampling site representing the shipyard area, whereas the highest mussel T-PAH concentration (201 ng/g) was determined at a site situated at the middle part of the Strat at the mouth of a creek. The lower amounts of T-PAH concentrations in mussels were detected compared to passive samplers. The maximum sediment (23620 ng/g) and local mussel (881 ng/g) T-PCB concentrations were measured at the middle part of the Strait. In general, the differences in the T-PCB concentrations of the measured sites were not significant. This could be due to the PCB compounds not being metabolized as the PAH compounds by the mussels. The accumulation of PCBs by the BR sorbents were not efficient as in case of PAHs. When SPMDs and BR sorbents were compared, the accumulation of PCB congeners in SPMDs were found much higher compared to the BR sorbents. OCP concentrations were only measured in sediments and mussels. The highest concentrations were measured at shipyard area. The most dominant OCP compounds were HCH and DDT derivatives. DDT concentrations in sediments were higher compared to mussels.

EC01A-2
Equilibrium sampling of environmental pollutants in Baltic Sea sediment along a transect in the Stockholm Archipelago
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The determination of the fugacity, freely dissolved concentration (Cf) or chemical activity of environmental pollutants in sediment is important since sediment is the dominant exposure medium for bottom-dwelling organisms and therefore is particularly relevant in a bioaccumulation context. Further, sediment represents the main reservoir for numerous hydrophobic organic chemicals (HOCs) in the aquatic environment, and the chemical activity in the sediment can govern the chemical activity in the water column.

To measure Cf of HOCs such as polychlorinated biphenyls (PCBs), a polymer is brought into contact with the sediment and analysed after equilibrium between the two phases is established. Here we used a coated glass jar with a very thin coating of polydimethylsiloxane (PDMS) on the vertical walls. The method makes use of three different coated glass jars (2 µm, 4 µm and 8 µm), which allows equilibrium between sample and PDMS to be confirmed for each sample and analyte (QA/QC), while at the same time verifying the absence of sampling artefacts.

In this study, we investigated Cf of PCBs in sediment samples from the Baltic Sea, collected along a gradient from central Stockholm towards the middle of the Stockholm archipelago. We determined the applicability of the coated glass jar method in the open Baltic Sea. The results showed a decrease in Cf with increasing distance from the urban center. This indicates that the Stockholm Waters continue to be a source of PCBs to the Baltic Sea close to four decades after the PCB ban in the 1970ies. The gradient could be caused by either ongoing releases to water or PCB residues in sediments from past emissions.

Further, we calculated PCB concentrations in biota (Cbw) and Kf values that express the sorption strength of PCBs to the different sediments. These data were compared to values from the literature and the spatial distribution of Cbw and Kf was examined. In summary, passive equilibrium sampling using coated glass jars was shown to be a convenient, accurate and sensitive technique to determine Cf of PCBs in Baltic Sea sediments.

EC01A-3
Effects of flow velocity and calibration conditions on a passive sampler for perfluorinated alkyl carboxylates and sulfonates in water
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Perfluorinated chemicals (PFCs) are emerging environmental contaminants with a global distribution. Due to the moderate water solubility of some PFCs, the majority of the environmental burden is in the water phase. Passive sampling provides a low cost and time-intensive sampling approach that has already proven useful for a broad range of environmental contaminants. A newly developed and validated Polar Organic Chemical Integrative Sampler (POCIS) with a weak anion exchange sorbent has shown potential as a passive sampler for PFCs in water. However more work was required to further validate the sampler. The aim of this work was to evaluate the influence of flow velocity and calibration conditions on the uptake of PFCs into POCIS sampler. Uptake kinetics and sampling rates for PFCs did not vary significantly with flow velocity. Sampling rates derived (0.08 – 0.28 L day⁻¹) are comparable to sampling rates determined in a previous study under different conditions. A passive sampler for PFC and similar compounds could help elucidate potential aquatic exposure routes to PFCs.

EC01A-4
Occurrence and fate of brominated and organophosphorus flame retardants in river water
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Perfluorinated chemicals (PFCs) are emerging environmental contaminants with a global distribution. Due to the moderate water solubility of some PFCs, the majority of the environmental burden is in the water phase. Passive sampling provides a low cost and time-intensive sampling approach that has already proven useful for a broad range of environmental contaminants. A newly developed and validated Polar Organic Chemical Integrative Sampler (POCIS) with a weak anion exchange sorbent has shown potential as a passive sampler for PFCs in water. However more work was required to further validate the sampler. The aim of this work was to evaluate the influence of flow velocity and calibration conditions on the uptake of PFCs into POCIS sampler. Uptake kinetics and sampling rates for PFCs did not vary significantly with flow velocity. Sampling rates derived (0.08 – 0.28 L day⁻¹) are comparable to sampling rates determined in a previous study under different conditions. A passive sampler for PFC and similar compounds could help elucidate potential aquatic exposure routes to PFCs.

EC01A-5
Porowater profiles of As, Fe, Mn, V and P in spiked marine sediment measured using DGT and DET techniques
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Arsenic and selenium are anionic contaminants, released into aquatic environments from the mineralisation of naturally-rich ores and industrial discharges. A diffusive gradients in thin films (DGT) technique to measure porowater profiles of As, Fe, Mn, V and P in spiked marine sediment is described. The study evaluates the performance of DGT and DET, precipitated ferrhydrite and titanium dioxide (Metsorb®) binding layers in sediments that were sieved (<1 mm), aged for 8 months.

In a second step, a monitoring study was performed along the River Aire, in Yorkshire, Northeastern England using grab samples and the passive sampler. In this latter case, unfiltered waters were extracted by means of solid phase extraction (SPE), through OASIS HLB cartridges. In grab samples, TCPP was the most abundant compound, followed by TCEP, TDCP and TPhP. Analysis was performed by gas chromatography coupled to mass spectrometry (GC-MS). As a first step, a passive sampler design based on a ceramic dosimeter and HLB as receive phase was tested and calibrated in laboratory conditions to determine uptake kinetics of a set of six flame retardants. The sampling rate obtained for TCPP, TCEP, TDCP and TPhP was 0.68, 0.31, 0.10 and 0.15 mg day⁻¹ respectively while lower values were found for the rest of FR, such as for HBB and PBB (0.4 and 0.6 ml day⁻¹, respectively).

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EC01A-6

Polar microsized micropollutants on SPE materials - which factors control the adsorption?


KWR, Nieuwegein, Netherlands

The toxicity data of all tested mixtures was successfully fitted to one chemical activity response relationship. The lethal chemical activities (La-50) of the individual PAHs and EC01B-3

Stimulation and Inhibition of bacterial growth by caffeine dependent on antibiotics and silver nanoparticles - a ternary toxicity study using a microfluid segment technique


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Hans-Knoell-Institute, Department Bio Pilot Plant, Jena, Germany

A droplet-based micro segmented flow technique was developed to fast generation of three dimensional concentration spaces for the screening of toxic effects of selected antibiotic substance and silver nanoparticles on the toxicity and activation of bacterial growth by caffeine at the nanoliter scale was introduced. Up to 1200 well separated fluidic compartments containing 216 different combinations of concentrations were realized in a single experimental run. To evaluate the toxicity of the ternary mixtures a time resolved miniaturized optical dual endpoint detection unit using a microflow-through fluorometer and a channel microflow-through photometer were used for the simultaneous analysis of changes on the endogenous cellular fluorescence signal and the cell density of E. coli cultivated inside 500 nl microfluid segments. As a result, a complex response pattern was discovered including synergistic and compensatory effects as well as strong non-linear combination effects, concentration dependent stimulation and the formation of activity summits on isobolographic maps. The results reflect a complex response of growing bacterial cultures depending on the combined effectors. A strong caffeine induced enhancement of bacterial growth was found at sublethal chloramphenicol and sublethal silver nanoparticle concentrations. The reliability of the method has been proven through the high redundancy of fluidic experiments. The results indicate the importance of multiple-paramater studies and prove the potential of the microsegmented flow technique for analyzing combined effects.

EC01B-2

Applying novel passive dosing systems for the control of chemical and drought stress to a terrestrial invertebrate

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Two passive dosing systems were used in this study to control (1) chemical stress and (2) combined chemical and drought stress in toxicity tests with the terrestrial springtail Folsomia candida. F. candida lives in the interstitial air in the top soil and is very sensitive to low humidity. The test compounds were the PAHs naphthalene, phenanthrene and pyrene. The aims of the studies were (1) to link lethality of naphthalene, phenanthrene, pyrene and their mixtures to (sum) chemical activity and (2) to determine springtail lethality in a two-factor experiment with both chemical and drought stress. Chemical activity is a multimedia parameter that expresses the energetic state of the contaminant. PAH loaded silicone elastomer was used to control defined chemical activities of the test compounds in glass vials. To start the 7-days test, 10 springtails were transferred to each loaded vial. F. candida could move freely on the loaded silicone, resulting in exposure through direct contact and headspace. In the test with combined chemical and drought stress, the chemical exposure was controlled by passive dosing, as described above. Passive dosing vials were covered with a net and placed inside a closed glass jar with aqueous saline solution in the bottom which controlled the humidity within the entire jar. In this way, F. candida was exposed to controlled chemical stress from below, while the drought stress was controlled from above. The PAH exposure parameter was in all cases chemical activity (untisless, [0-1]), and the humidity was expressed as relative humidity, which is also known as water activity. The two passive dosing systems were simple and practical to use, and the systems worked well with high survival in controls and low variation in lethality within treatments. Springtail lethality caused by naphthalene, phenanthrene and pyrene was successfully linked to chemical activity. Springtail lethality caused by four PAH mixtures (each in three dilutions) was plotted as a function of sum chemical activities, and the toxicity of the chemical mixtures was successfully predicted by activity response relationship. The lethal chemical activities (La-50) of the individual PAHs and the mixtures were all well within the expected range of 0.01-0.1 for baseline toxicity. The results from the combined stressors experiment showed stress synergy in springtail lethality between the chemical and drought stress.

EC01B-3

Passive dosing under the microscope reveals that microorganisms enhance the mass transfer of hydrophobic organic chemicals

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The transport of hydrophobic organic chemicals (HOCs) is often rate limited by their diffusive mass transfer through stagnant boundary layers (SBLs). This then results in gradients on the microscale, which are crucial for the diffusive transport of HOCs and for organisms living in such boundary layers. To study how microorganisms react to such gradients, and whether they can alter the transport of HOCs through SBLs, a new experimental setup was developed. In this setup, microgradients of HOCs could be precisely controlled and quantified. At the same time microscopic observation of organisms exposed to the gradients could be performed. Passive dosing was employed on microscope slides to produce gradients of polyaromatic hydrocarbons (PAHs) by integrating silicone O-rings into the Dunn Chemotaxis chamber. An outer clean ring thereby served as a sink and an inner PAH-loaded ring of the same source. The mass transfer of the PAHs from source to sink by diffusion through protozoa medium was then quantified, and compared to that of the chemical mixtures of the ciliate Tetrahymena pyriformis. The active uptake and release of PAHs by T. pyriformis performed the mass transfer was enhanced, increasing with the hydrophobicity of the PAHs. This enhancement was nearly hundred-fold for benzol[α]pyrene, the most hydrophobic compound tested. Fluorescence video imaging microscopy showed that this enhancement was based on a mechanism where protozoa acted as a transport vector of PAHs via their diffusive uptake and release. Such a transport mechanism has, to our knowledge, not yet been described.

EC01B-4

Comparison of two monitoring strategies for organic pollutants in seawater: passive sampler devices vs transplanted clams


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The concentration of organic pollutants in seawater shows a great temporal and spatial variability as a result of a combination of natural and anthropogenic effects. In this study we have characterized simultaneously the bioaccumulation in transplanted clams and two passive sampling devices (semipermeable membrane device, SPMD, and continuous flow integrative sampler, CFIS). The combination of these monitoring techniques such as water, biota and passive samplers with the analysis of water concentrations allows a comprehensive knowledge of the environmental status and to assess the representativity of these integrative sampling methods. The efficiency of these two passive samplers and the determination of the bioaccumulation of organic pollutants in transplanted clams were tested in the marine environment in spring and autumn comparing the obtained integrative concentration with the real water one. The study was performed in four selected sampling sites in Mar Menor Lagoon (SE of Spain), which is subjected to direct and indirect discharges of organic pollutants. El Albujón Wadi is the main surface watercourse that flows into this lagoon from Cartagena Field, which is one of the most relevant horticulture areas in Europe. The organic pollutants determined in clam were PAHs, PCBs and organochlorinated pesticides. In surface and marine waters PAHs, PCBs, triazines, organophosphorus and organochlorinated pesticides were analysed by stir bar sorptive extraction and thermal desorption coupled to capillary gas chromatography-mass spectrometry (SBSE-TD/GC/MS). Significant daily, weekly and seasonally differences were observed in the input of organic pollutants. Chlorpyrifos ranged from 0.9 to 12 ng/L in spring and from 3 to 6000 ng/L in autumn. Duplicate and triplicate of CFIS and SPMD were studied in several sampling points in
order to study the repeatability (RSD>20%) of passive samplers. Chlorpyriphos was detected in spring in all cases, both in SPMD (4.8-130 ng/L), showing a great variability, and CFIS (4.68-17.25 ng/L) this insecticide only detected with CFIS (2-18 ng/L) in autumn. An increase of concentrations in clams for pesticides and PCBs were observed after a month of exposure in the mouth of El Albuñuel watercourse. In general both in Spring and Autumn the intermediate exposure time was too short to reach similar pollutants levels found in CFIS or SPMD.

EC01B-5
A passive sampling method for the estimation of concentrations in pore water and accessible concentrations of polycyclic aromatic hydrocarbons in contaminated sediments

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For the environmental risk assessment of contaminated sediments it is important to know both the equilibrium concentrations in the pore water (C\textsubscript{w}) and the accessible concentrations in the sediment (C\textsubscript{s}). C\textsubscript{s} is highly relevant for uptake by organisms and transport, and the accessible concentrations in sediment are important for distinguishing between the contaminant fractions that may be subject to bioaccumulation and biomagnification on one hand, and fractions that are strongly bound to resistant sediment constituents, such as soil and coal, on the other. Traditional methods for determining accessible concentrations are extraction of sediment suspensions for a fixed period of time using gas purge or TENAX. Concentrations in the pore water traditionally are measured in sediment suspensions by passive sampling (e.g. SPME, polycarbonate membrane, low density polyethylene or silicone rubber), assuming that uptake by the passive sampler does not substantially lower the initial C\textsubscript{w}. We developed a silicone passive sampler based method for the simultaneous determination of C\textsubscript{w} and C\textsubscript{s} by incubating sediment suspensions at increasing silicone/sediment phase ratios. This method allows for determining sediment-water sorption isotherms, because higher silicone masses cause a lowering of the concentrations in both the sediment and the pore water. Sediment samples from 3 different areas in the Netherlands were equilibrated with silicone samplers by shaking at 150 rpm for 28 days with 4.5 L of water. The silicone sorption isotherms ranged from 0.03 at a suspension density of 0.1 g/mL. The ratios show that the accessible contaminant fraction follows linear sorption isotherms, and that only 10.25% of the total concentrations can be released from the sediment to the aqueous phase on the timescale of the experiments. The results are highly relevant for the risk assessment of contaminated sediments.

EC01B-6
Evaluation of an in-situ equilibrium sampling device for persistent organic pollutants in sediment pore water systems on the basis of solid phase microextraction (SPME)

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In this study, equilibrium passive sampling device is introduced that makes POP bioavailability in terms of freely dissolved aqueous concentrations assessable on site, i.e. in the environment. The calibration step showing capability of POPCs allows for in-situ or solvent-based sample extractions that make it an ideal monitoring tool:
(1) The ecologically relevant parameter ‘in-situ bioavailability’ is addressed instead of total sediment or pore water concentrations,
(2) Due to short/medium equilibrium times, the temporal resolution of the measurements is suitable for analysis of both long-term trends and seasonal effects.
(3) The device is of very solid construction and can be reused practically ad infinitum; only replacement of the disposable sampling materials contributes to its operational costs.
(4) Sample treatment is reduced to a minimum which in turn reduces possible sources of sample manipulations, measurement errors and analysis costs. Both sampling and analysis procedures are thus simple, robust and cost-effective.

The device is already applicable in a multitude of aquatic environments, especially where currents are low and sediments are muddy and well-mixed e.g. by bioturbation. Examples for such environments are mud flats, harbor basins, river banks and lakes.

EC01C-1
Use of plants as passive samplers for volatile organic compounds (VOCs) in indoor environments

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Concerns about the potential build up of volatile organic compounds (VOCs) in indoor air have increased as energy conservation methods minimized the introduction of outdoor air. VOCs, including many with documented short- and long-term adverse health effects, can be enter indoor environments through internal (i.e. paints, carpets, cleaning supplies, pesticides, building materials, adhesives) and external sources (i.e. vapor intrusion from contaminated groundwater). Indoor air concentrations of VOCs vary widely, but concentrations of most VOCs are consistently higher indoors than outdoors. Typical approaches used to sample indoor air include evacuated canisters and sorbent tubes. The use of ornamental plants has been suggested as a simple, nonobtrusive, aesthetically pleasing, and cost effective method for sampling and purifying indoor air. The waxy surface of the leaves has the potential to provide a good surface for the passive capture of VOCs. However, the efficiency and kinetics of capture has not been well characterized. To investigate the potential use of plants as indoor air VOC samplers, three types of studies were performed. The first consisted of monitoring air and plant concentrations over time after a controlled release of several VOCs into a residential building containing several plant species. The second study used a flow-through glass and stainless steel plant growth chamber to evaluate the relationship between air and plant leaf VOC concentrations. The third study used a headspace approach to measure equilibrium leaf-air partition coefficients. Good correlations between the leaf and air concentrations observed in the three different studies suggest that plant leaves can be used to monitor indoor air concentrations of VOCs.

EC01C-2
Calibration and field evaluation of Polar organic chemical integrative samplers (POCIS) for monitoring pharmaceuticals in hospital sewage water

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Pharmaceuticals have become an important issue in the last few years due to their continuous release into the environment. In this context, we made the MEDIFLUX study to evaluate the contribution of hospital to the urban sewage pollution by pharmaceuticals. To go on, we decided to test Polar Organic Chemical Integrative Samplers (POCIS) for passive sampling of hospital sewage water. Passive samplers allow the measurement of Time Weighted Average (TWA) concentrations, overcoming many shortcomings of the spot sampling techniques. The application of POCIS was studied using six compounds already selected as representative of the great families of pharmaceuticals used at the hospital (Atenolol, Prednisolone, Methylprednisolone, Sulfamethoxazole, Olfaxcin, Ketoprofen). In a first step, POCIS were calibrated in tap water under laboratory conditions for the analytes of interest taking into account various relevant environmental conditions (temperature, flow rate...). POCIS were exposed under a constant agitation to tap water spiked with the selected pharmaceuticals, renewed every two days. Sampling rates were determined and compared. It appears that Rs increase significantly when flow rate increase between 10 to 25 cm.s\(^{-1}\) for all the compounds (except sulfamethoxazole). A slight increase of Rs was observed for sulfamethoxazole.

In a second step, the flow rate and temperature of water were reduced during a week on the selected hospital effluent. For the calibration, 20 L of wastewater were taken from hospital effluents and brought to the laboratory to carry out Rs measurement as for the previous measurement in tap water. Sewage water was changed every two days. The Rs values obtained were closed to Rs values in tap water. The step of calibration was the main part of the work and its completion was needed before the application of POCIS in situ. The follow-up of accumulation made it possible to estimate the field of linearity in order to choose the optimal length of POCIS implantation in situ. The use of POCIS for the follow-up of contamination by organic pollutants is especially described for surface waters such as rivers, but very little studies report its use in wastewater. This work gives encouraging results for the deployment of POCIS in sewage that could be a useful tool for pharmaceutical pollution management.

EC01C-3
Accumulation kinetics and sampling rates for 56 polar organic compounds, identification and validation of 5 PRCs

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\textsuperscript{3}POCSYS (Paris Organic Chemical Integrative Sampler) is a new emerging tool for sampling polar organic micropollutants in water. There is still a need of research concerning its domain of validity (e.g., molecules sampled, type of water studied, optimal exposure duration) and its performances (molecules sampling rates, repeatability, accuracy of the evaluation of time-weighted average or TWA concentrations). In order to obtain in situ TWA concentrations, POCIS needs to be calibrated in laboratory for each molecule of interest. We performed 3 different laboratory calibration experiments using a flow-through system in order to establish the optimal exposure duration and to calculate the sampling rates for 56 polar organic contaminants, and to identify and validate performance reference compounds (PRCs). The calibration system was composed of 2 aquaria (50 L) containing i) spiked tap water (circa 3 µg/L) and non-spiked POCIS for the determination of accumulation kinetics, ii) non-spiked tap water and spiked POCIS for the determination of desorption kinetics and iii) spiked tap water and spiked POCIS for the validation of the potential PRCs. In all experiments, water temperature, pH, conductivity and dissolved organic carbon were controlled. Agitation in the aquaria was ensured via a submerged pump. The resulting flow velocity was 10.5 cm/s and was directed perpendicularly towards POCIS surface. Tripleyne POCIS were analyzed at 0, 1, 3, 6 and 12 hours and at 1, 3, 7, 11, 14, 21 and 28 days.
ECO2-1  Contaminant pathways, trends and biological effects in a warmer Arctic

ECO2-1 Influence of climate change on contaminant distribution and effects in Arctic marine food webs - Summary of the JPY project COPOL
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An overall aim of COPOL was to evaluate and quantify the seasonal and annual variation in bioaccumulation and effects of contaminants (organic contaminants and trace elements) in benthic and pelagic Arctic marine food webs. This was important in order to be able to affect the climate of a signal from other expected system variability. Two fjords on Svalbard with different climate conditions were selected as study sites: Kongfjorden (Atlantic signal) and Liedefjorden (Arctic signal).

In summary, the seasonal variation in the pelagic food web was greater than both between year and between fjord variations. This was found both for absolute concentrations from zooplankton to fish and birds, and for the average concentration increase per trophic level (food web biomagnification estimated by trophic magnification factors). The high within year variability underlines the importance of coherent studies of specific time periods rather than pooling samples across a year. It also means that if studies should repeat annually, it is important to select one representative time period.

We presented the results for the 56 studied molecules, including the duration of linear accumulation phase, sampling rates and possible performance reference compounds (PRCs). The accumulation phase was curvilinear for almost all molecules; so, we obtained the duration of optimal linear accumulation phase using t1/2 criteria. Concerning the desorption experiments, 5 compounds showed a good potential as PRC; they enable to decrease the effect of variable environmental conditions, so they can be used to obtain more reliable in situ TWA concentrations. We also checked isolated exchange for these molecules comparing the exchange constant ku and a accumulation and desorption experiments. At last, we validated the PRC strategy by calculating TWA concentrations from third laboratory experiment mentioned above.
The impact of a changing climate on contaminant fate in the physical environment of cold regions is likely to be amplified by the sensitivity of the cryosphere to relatively small changes in temperature that will modify a contaminant’s environmental behaviour, but the changes in the extent and duration of a seasonal snow and sea ice cover and in the nature of the snow melt. In order to anticipate the potential influence of climate change, we seek to improve the general understanding of contaminant-cryosphere interactions through a combination of laboratory experiments, field studies and computer simulations. Snow is subjected to controlled melting in a cold room laboratory and the fractionated melt water is filtered and the sorbed and dissolved fractions analysed with appropriate trace analytical techniques. Elution curves for different contaminant groups of variable partitioning properties, for different types of snow and for different melting conditions are recorded. Field studies in a highly urbanised watershed involve the repeated sampling of river water throughout the snow melt season. Sorbed and dissolved fractions are analysed by analytical techniques. Elution curves of contaminants from a melting snow pack are predicted with a simple mass balance model that simulates the sequential melting of several horizontal snow layers and the resulting downward percolation of melt water. The model assumes equilibrium partitioning between the various snow pack phases. Laboratory experiments revealed at least five types of elution curves for organic contaminants from a melting snow pack. All types could be reproduced with, and thereby mechanically explained by, the snow pack melt model. Elution curves in river water do not resemble those eluting from laboratory snow packs, except that water soluble contaminants tend to appear early during the melt period. Particle-bound contaminant concentrations in the river tend to peak sharply during snow melt, and correlate with river run-off rates. Concentration time profiles of particle bound contaminants in rivers during snow melt are less controlled by processes occurring within the snow pack, and depend more on factors that determine run-off rate and the mode of melt water ablation from the snow pack to the stream. Many of these factors are influenced by a changing climate.

ECO2A-3
Climate change and Arctic marine mercury biogeochemistry - conclusions and research needs from the AMAP 2011 Arctic Mercury Science Assessment
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Recent studies have shown that climate change is already having significant impacts on many aspects of transport pathways, speciation and cycling of mercury (Hg) within Arctic ecosystems and ultimately biological exposure, including humans. For example, the extensive loss of sea-ice in the Arctic Ocean and the concurrent shift from greater proportions of perennial to annual ice have numerous effects including promoting increases in primary productivity, shifting food web structures, possibly altering methylation and demethylation rates, and influencing Hg transport and distribution across the ocean-sea-ice-atmosphere interface (i.e., bottom-up). In addition, changes in animal social behavior, such as habitat selection in association with changing sea-ice regimes by top predators such as beluga, can also affect dietary exposure to Hg (i.e., top-down). In this presentation, adapted from one chapter of the recent AMAP Mercury Science Assessment, we address these and other possible ramifications of climate variability on marine Hg cycling, processes and exposure in the Arctic.

ECO2A-4
Local contaminant sources in the Arctic: hysteretic and non-volatilize residues from combustion engines in surface soils from snow mobile tracks in the vicinity of Longyearbyen (Svalbard Norway)
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A summer field campaign was conducted in 2010 for investigating potential long-term effects on surface soils from fossil fuel burning. A total of 180 soil samples and two surface snow samples were collected for the analysis of polycyclic aromatic hydrocarbons (PAHs) along frequently used snowmobile tracks in front of Longyearbyen (78°13'0 N, 15°37'00 E). In addition, Benzo[a]Pyrene-Xylene (BTX) components were measured in ambient air at the University Centre in Svalbard Laboratory facilities during spring and in autumn 2010. Polycyclic aromatic hydrocarbons (PAH) was determined in all 18 soil samples in the concentration range 21 to 1883 ng/g dw (dry weight, SUM 15 PAH). All aspects in soil and the snow were characterized with pyrogenic PAH patterns. The highest concentrations were found in a sample from the former winter airfield close to the old Northern Light Observatory. Only 30% of the 2007 contaminations were detected for Benzene in 2010. Longyearbyen air. This significant concentration reduction is assumed to be caused by the higher proportion of 4-stroke engine driven snowmobiles compared to the 2010 season.

ECO2A-5
The impact of thawing permafrost on lakes of the Mackenzie Delta uplands, NT, Canada
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Total permafrost in the Northern Hemisphere currently occupies an area of 26 million km2, and by 2100, this area is expected to decrease by 19-35%. In the Mackenzie Delta, NWT, temperatures are projected to rise by 4 to 5°C in the next 50 years. Over the past 20 years, mercury and PCBs have been steadily rising in burbot from the Mackenzie River, which has prompted speculation on how the changing physical environment, such as thawing permafrost, might be affecting contaminant cycles in these temperature sensitive environments. We tested the hypothesis that the presence of retrogressive thaw slumps in the Mackenzie Delta Uplands (north of Inuvik, NT, Canada) is affecting nutrients (total and dissolved N and P), persistent organic pollutants, metal concentrations, and phytolankton community assemblages in small tundra lakes. Dissolved organic carbon, total phosphorus, soluble reactive phosphorus, and total and methyl mercury were significantly lower in lakes with retrogressive thaw slumps than reference lakes, likely due to deeper water infiltration through clay-rich tundra soils. These results provide compelling evidence that thawing permafrost near lakes of the Mackenzie Delta uplands are not responsible for the rising trend in mercury concentrations of fish in the Mackenzie River.

ECO2A-6
Burdens and inputs of perfluorinated compounds in the Lomonosovfonna Ice Core, Svalbard (2009)
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In 2009, we drizzled a 40-meter ice core representing ~1953 - 2008+ from Lomonosovfonna (78°49' 24.4' N; 17°25' 59.2'E), the highest elevation glacier on Svalbard (1250 m.a.s.l.). We sub-sampled the upper 21 meters of the core to identify inputs of 12 perfluorinated compounds (PFCs) delivered to the site by long-range atmospheric transport. Each sample had liquid volumes of 291 mL (average), and dating resolution averaged 2.4 years from 1976 - 2008. Five compounds were not detected in any segment of the core, including PFBA, PFHxA, PFHpA, PFBS, 6:2FTS. PFNA dominated the accumulated burden in the core, showing 29.4 pg cm-2, 81% more than the second highest, PFOA, 16.2 pg cm-2. It is thought that the PFNA and PFOA may be atmospheric oxidation products of 8:2 FTOH which has been found to be the dominant gas-phase PFC in Europe. However, it is also thought the PFNA may be the dominant PFC in the atmospheric phase in Europe; these results suggest that PFCs in both phases are reaching mechanistically explained by, the snow pack melt model. Elution curves in river water do not resemble those eluting from laboratory snow packs, except that water soluble contaminants tend to appear early during the melt period. Particle-bound contaminant concentrations in the river tend to peak sharply during snow melt, and correlate with river run-off rates. Concentration time profiles of particle bound contaminants in rivers during snow melt are less controlled by processes occurring within the snow pack, and depend more on factors that determine run-off rate and the mode of melt water ablation from the snow pack to the stream. Many of these factors are influenced by a changing climate.

ECO2B-1
The deposition and fate of perfluorinated alkyl substances (PFAS) in the Norwegian Arctic snowpack
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Polyand perfluorinated alkyl substances (PFAS) are man-made chemicals that are ubiquitous in the environment and are present in humans and wildlife. These chemicals occur in the Arctic through long-range transport processes with the perfluorinated acids (PFAAs) [e.g. carboxylates (PFCAs) and sulfates (PFSAs)], present in sentinel
organisms such as the Polar bear (Ursus maritimus) and the ringed seal (Phoca hispida). PFAS are present in arctic media through a number of processes, including transport with ocean currents, oxidative degradation of 'neutral' precursors (e.g. fluorotelomer alcohols) in the atmosphere and possibly transport with airborne particles and aerosols. A combination of these processes results in their deposition with snowfall, although only a few studies to date have examined accumulation of these chemicals in the Arctic snowpack. Questions arise regarding the sources of these chemicals in atmospheric deposition in remote locations and the role of snow in delivering PFAS to catchment systems during periods of melt. In this study, a detailed examination of PFAS was undertaken in the seasonal snowpack at remote terrestrial sites in Northern Norway. The purpose was to investigate, in some detail, their accumulation in different snow layers and to relate their profile and concentrations to physical and chemical changes of the separate snow layers as well as their accumulation history. PFAS measurements in air (gas phase and particle-bound) and snow (dissolved and particulate matter) were considered. Air and snow samples were collected to examine air mass origins for specific air samples and snowfall events. In the snowpack, the vertical evolution of snow density, snow layer hardness, crystal morphology (macrophotography) was examined alongside temperature, pH, conductivity, organic matter content, major anions and cations, as well as particle characteristics, to help understand sources of PFAS to fresh snowfall and their fate during snow aging. Initial results show large variations of pH and particle matter content between snow layers, with pH measurements as low as pH 4.68 in some layers, indicating the potential for a fraction of the PFAS to exist in their neutral acid form and hence be 'remobilised' during thaw periods.

**ECO2B-2**

Secondary emissions of legacy pollutants and their pathways to the Arctic under a changing climate

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The phase-out of a persistent chemical can be expected to endure in the environment for some time, up to several decades. How long and where it will persist depends on its emission history in transport, partitioning between environmental compartments, and degradation. With respect to the accumulation of legacy pollutants the Arctic is a particularly interesting region. It is also in the Arctic, where future climate change is expected to affect most the presence of persistent pollutants. The mechanisms leading to a particular Global distribution of the contaminant are addressed by multimedia chemical fate models. These models provide not only quantitative estimates of the global distribution, but also an understanding of the pathways involved, and allow forecasts under different emission and climate scenarios. In this work we model concentrations of some representative persistent organic pollutants in the Arctic after their phase out. In particular we indicate the timescales that can be expected for those chemicals to persist in the Arctic, and analyze the relationship between Arctic receptor sites and global secondary sources. Finally, we investigate the effect that the projected climate change may have on levels in the Arctic and the global distribution of secondary sources.

We show that beta-HCH and PCB153 will persist in the Arctic Environment for several decades after cessation of primary emissions with apparent half-lives of about 8 and 28 years, respectively. These results are only slightly sensitive to forecasted climate change. We further demonstrate that the location of secondary sources important for the Arctic is strongly dependent on the chemical properties of each pollutant.

**ECO2B-3**

Spatial and temporal patterns of persistent organic pollutants and mercury in ringed seals from the Canadian Arctic

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The goal of this ongoing study is to determine temporal trends of legacy POPs, new/emerging POPs, as well as mercury (Hg) in Canadian Arctic ringed seals using annual collections at Aviat, Resolute, and Sachs Harbour, as well as less frequently at nearby communities. The study builds on results for legacy POPs and Hg going back to the 1980s, and earlier in some cases. Results for PBDEs, HBCD, perfluorinated chemicals (PFCs), and endosulfan, along with δ13C and δ15N data, have been added to samples collected since 2001 and on selected archived samples from the 1970s and 1990s. Sample collections (10 to 25 adult ringed seals, blubber, liver, muscle, tooth/flower jaw for aging) are carried out by local hunters each year (June-October). For neutral POPs, only blubber of females and juveniles are analysed to limit the influence of age. For Hg, muscle and liver samples are analysed, while PFCs are also determined in liver. Highest Hg concentrations in seal muscle and liver (age ≥ 5 years) were found in western and central Arctic. No significant increase or decline of Hg in seal muscle was found over a 7 to 9 year period at Aviat, Resolute and Sachs Harbour. δ13C and δ15N in seal muscle varied only over a narrow range (±0.5 %) indicating little change in diet over the same period. Hg concentrations in liver were more variable over the same period. This was not related to diet or to age but could reflect recent diets of individual animals compared to measurements of muscle N. To assess time trends of legacy and new POPs we combined data for nearby communities. Overall, there are declining trends in all regions with the relative magnitude of ZDDT:di-HCH:2,4,5-TPCB:2,4,5,6-TCB. Largest declines of all legacy POPs were in Hudson Bay possibly reflecting proximity to source regions in North America. PBDEs, PFOs, and PFCs show increasing concentrations in the 1990s to early 2000s and then recent declines. Endosulfan and HBCD were present at low concentrations in seal blubber (0.01-2.0 ng/g) and appear to be increasing in concentration. Trends analysis were not done for the 2005-2010 period. Temporal trends of legacy POPs in the Canadian arctic generally show declining trends. However trends for new POPs differ from those in Greenland particularly for PCBs. Continued annual sampling is improving the statistical power of the study and enabling testing of factors influencing trends of POPs and Hg including climate, diet and changes in global emissions.

**ECO2B-4**

An observation-based mass balance assessment of PCBs in the Arctic Ocean

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The predictability of future distribution and exposure of PCBs in the Arctic hinges centrally on the quality of our understanding of the present-day distribution and processing of PCBs in the Arctic system. Real observations of PCBs in abiotic compartments in the Arctic Ocean are scarce, increasing the importance of model simulations for understanding the fate of PCBs in this extreme but sensitive environment. The objectives of the current study were to a) present an observation-based inventory of PCBs in the Arctic Ocean (AO), b) to relate observations to total global emissions and modeled simulations of transport to the Arctic, offering an assessment of how well we understand transport and fate of PCBs in the Arctic today, and c) to present a mass balance for the entire AO, identifying the major inventories, import and export fluxes of PCBs. Water samples were collected covering all the water masses of the central AO (CAO) and surface water from the polar mixed layer in all seven shelf seas. Observed concentrations in combination with annual global emissions for the years 1930-2000 were used to estimate concentrations back to 1930. The total PCB77 inventory for all Arctic waters was estimated to be 6500 t, but also an understanding of the pathways involved, and allow forecasts under different emission and climatic scenarios. Furthermore, this work shows that the Arctic AO of approximately 25 years allowed this water to accumulate PCBs. Hence, water from the Atlantic layer exported from the AO contains higher concentrations of PCBs than water that entered the AO, potentially offering a partial explanation for the discrepancy between model simulations and observation-based calculations presented here.

**ECO2B-5**

Exposure of persistent organic pollutants in avian top predators in a changing northern climate

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Top predators in the Arctic ecosystem are the final destination of bioaccumulative POPs and mercury, and in such organisms the negative impacts are most likely to occur. However, little is known about how climate change will affect the accumulation of POPs in top predators. Changes in POPs in Arctic top predators may occur by two major processes: 1) through increased POP transport or 2) indirectly through changes of POP uptake in the food chain and changes in diets of top predators. The importance of these pathways is little understood, and the aim of this presentation is to discuss how changing climate and feeding ecology may affect the uptake of POPs in different species of top predators. Relationships are shown to factors both related to POP transport and to indirect mechanisms such as local climate and feeding conditions.

**ECO2B-6**

Persistent organic pollutants (POPs) in Svalbard water foods: is the Arctic far enough?

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Persistent organic pollutants (POPs) are semi volatile and have high environmental half-lives. These characteristics produce a long-range atmospheric transport and global planetary distribution, reaching locations far away from the pollution source. The overall process results in an enrichment of concentrations of some POPs in polar ecosystems, which became a global sink due to their environmental conditions. As a consequence of their biomagnification properties, high trophic food webs organisms...
provide a great opportunity for the analysis of background concentrations of POPs accumulated in Arctic biota. Two water ecosystems from Svalbard archipelago (Arctic) were selected encompassing freshwater and marine fish as a targets (summer 2010). 18 Arctic char (Salvelinus alpinus) and two local marine fish species (17 Cod (Gadus morhua) and 9 Haddock (Melanogrammus aeglefinus)) were sampled.

Traditional persistent organic pollutants (PeCB, HCB, HCHs, DDTs, PCBs) were analysed in all samples and recently introduced compounds such as the polybrominated diphenyl ethers (PBDEs) have also been assessed. Arctic char showed higher heterogeneity in the abundance of groups of POPs (83-95% for PeCB, HCB, HCHs and DDTs and 50% for PCBs) while in marine species these compound groups were found in 100% of the samples. Mean concentrations of these first 5 groups ranged between 0.4 and 17.8 ng/g. Higher values were found for HCHs in all species. This could be explained by the high solubility and volatility of these compounds in relation to other compounds. For PeCBs, the highest concentration was observed in Arctic char and in 35% of marine samples. Concentrations of total PBDEs ranged between 0.004-0.306 ng/g, 0.010-0.177 ng/g and 0.005-0.538 ng/g in Arctic char, Cod and Haddock, respectively. The most abundant congeners were PBDE-47, PBDE-99 and PBDE-209.

The results from our work confirm a background level of organochlorine compounds in Arctic regions. Further studies should also include other emerging organic compounds of concern in freshwater and marine biota.

**EC04 - Novel approaches to addressing metal and metal nanomaterial bioavailability in soils**

**EC01-4** Validation and first deployment of the DGT technique in artificial human gastrointestinal fluids after ingestion of metal-containing soil particles

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The accumulation of metals in soils due to human activities constitutes a potential health risk if directly ingested, especially by children via hand-to-mouth behaviour. Contaminants can be partially or totally released from soil by ingestion, depending on their speciation under gastrointestinal conditions. In vitro tests provide estimates of bioaccessibility, defined as the proportion of contaminant that is dissolved in the artificial gastrointestinal fluids and is potentially available for absorption. The flux toward the gastrointestinal membrane corresponds to both the free metal ion and labile metal species. In contrast, inert species cannot dissociate and thus do not contribute to transport across the intestinal membrane. Estimation of the labile fraction can be assessed with the Diffusive Gradient in Thin Films (DGT) technique. Whereas the effectiveness of DGT has been demonstrated for various metals (Cd, Zn, Cu, Ni, Pb) in different exposure media (natural waters, soils and sediments), no data is available in artificial human gastrointestinal fluids. The objectives of this study were firstly to validate the performance of the DGT technique for Cd, Pb and Zn in controlled digestion solutions for different times of exposure and different metal concentrations, and secondly to use the technique in the gastrointestinal solutions obtained after carrying out the in vitro Unified Barge Method (UBM) test on highly contaminated soils. The results demonstrated the suitability of the DGT technique for Cd, Pb and Zn measurements in the gastrointestinal media with linear response and stable accumulation up to 6 hours at 25°C and then at 37°C. Combining the in vitro test with the DGT technique provided an approach to the labile metal species available for transport across the intestinal epithelium. Thus, the gastrointestinal absorption of ingested metals ranged from 8% to 30% for Cd, 11% for Pb, and 0.4% to 7% for Zn and was influenced by metal speciation. In this original approach, the DGT technique was found to be simple and reliable in the investigation of labile metal species in digestive fluids. Extrapolation to the in vivo situation should be undertaken very cautiously and requires further investigation.

**EC01-2** Comparison of the determination of free Zn(II) concentration in soils using AGNES and DMT

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Key paradigmatic soil and environmental sciences, such as the Free Ion Activity Model (FIAM) or the Biotic Ligand Model (BLM), attribute a central role to the free metal ion concentration (rather than total metal concentration) in the toxic or nutritional effects of a particular element. Consequently, there is a significant interest for analytical techniques that can act as selective probes for the free Zn(II) concentration in a large variety of environmental systems, and, specifically, in soils. The measurement of free Zn(II) concentration is particularly challenging, because there is no commercial Ion Selective Electrode for this element. Only a few techniques have a direct access to the free metal ion concentration, while many other popular techniques (such as DGT, Diffusion Gradients in Thin Films) measure operationally defined fractions (e.g. a certain labile fraction). AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) and DMT (Donnan Membrane Technique) provide robust and direct measurements of the free metal ion concentrations. In the literature, AGNES has been applied for the determination of free Zn in seawater, freshwater, humic acid solutions, ZnO nanoparticles dispersions, etc. DMT has been applied to soils, natural waters, etc.

In this work, the application of both techniques to the same synthetic and natural systems allows a cross-validation. AGNES and DMT are validated in synthetic solutions of Zn-NTA, yielding results in agreement with each other and with the theoretical code VMINTEQ. A further validation came from analysis of River water. A critical comparison of the characteristics of these techniques can be performed in terms of time of analysis, limit of detection, required instrumentation, etc.

Thus, the DGT technique and AGNES are complementary techniques and can be used to determine the free Zn concentration in different types of soils extracts. The analyses of free Zn in 4 soil extracts (river clay, cover sand, loam and reclaimed peat; all from the Netherlands) also gave similar concentrations with both techniques, and consistent with ECOSAT theoretical predictions. The impact of the small pH drift in DMT donor solutions is negligible. The percentages of free Zn (with respect to the total zinc concentration) range between 40% and 80%. This indicates that, in the four considered soils, Zn is much less bound than Cu, which, in principle, should imply a larger bioavailability of Zn (II) to plants and animals.

**EC01-3** Using radioactive and stable metal isotopes to study metal and metalloid availability and ecotoxicity in soils

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Methods to study the speciation of metals in the environment has progressed rapidly over the last few decades, with the principal advances being in aqueous phase methodologies. In soils (and sediments), speciation of metals in the solid phase is problematic, with classical sequential fractionation schemes being operationally defined and having several drawbacks. Synchrotron x-ray spectroscopy has opened up new avenues to examine solid phase speciation of metals in soils, but suffers from the drawback that it cannot quantitate metal availability and behaviour, which must be inferred from knowledge of solid-phase forms identified. Isotopic methods can be used to either trace metal/metalloids in particular forms added to soils, or isotopic dilution can be used to examine the fate and behaviour of materials that cannot easily be isotope-labelled (e.g. manufactured materials or wastes).

Isotopic methods provide an extremely valuable tool to probe the fate, biotransformation and availability of metals and metalloids in soils. The information provided by isotopic methods has already been used in regulatory frameworks for metals risk assessment and will continue to be a vital tool in probing metal and metalloid behaviour in soils. Looking to the future, new methods examining the differences in the natural abundance of metal/metalloids isotopes abundance will open up our understanding of metal/metalloid availability in soils.

**EC04-4** Stable isotopes for micronutrient metal bioavailability to earthworms

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In urban environments two of the most common metal contaminants are copper and zinc. In addition to being contaminants, these metals are regulated micronutrients in most organisms. Both have stable isotopes (64Cu and 68Zn) that are readily quantified and differentiated from the more abundant isotopes (63Cu and 66Zn) by modern instrumental techniques (ICP-MS). There are two ways to use stable isotope techniques in this context: first, we hypothesized that by modifying the metal isotopic ratio in a soil, we could elevate the ratio of 65Cu/64Cu or 68Zn/Zn in an earthworm (either Eisenia fetida or Lumbricus terrestris) and also assess whether the ratio changed in that ratio after placing the worm into a soil with a normal isotopic ratio could be used as a metric of bioavailability. The change in ratio would be a result of either an exchange of stable isotope for the more abundant isotope or the dilution of the ratio due to accumulation of the abundant isotope; in either case the ratio, the change in that ratio after placing the worm into a soil with a normal isotopic ratio could be used as a metric of bioavailability. The change in ratio was a result of either an exchange of stable isotope for the more abundant isotope or the dilution of the ratio due to accumulation of the abundant isotope; in either case the ratio would decrease in the worm where the Earthworm could accumulate the metal. Our research using stable isotopes has shown usefulness in determining micronutrient metal bioavailability in soils. In both Cu and Zn, isotopic ratios were increased 6-8 fold over background ratios by exposing worms to labeled soils. When placed back into soils with natural isotopic abundances ratios returned to near background levels. Understanding differences in how organisms store and utilize these metals is important for how these methods are to be further developed. Zinc isotopes show promise due to its longer term storage in the anterior organ tissues of L. terrestris, while the extremely fast turnover rate of copper in E. fetida suggests that stable isotopes may not be as useful as first hypothesized.

**EC04-5** Weathering of silver nanoparticles could increase their bioavailability

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The major challenge in tracing engineered nanoparticles (ENPs) in complex media, such as soils, is to detect their presence, transfer to organisms and their interactions with SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting 27
AgNP soils

At the end of the exposure period (day 28), earthworms had body concentrations corresponding to 5.1 ± 0.5 % and 11.0 ± 0.3 % of the concentration in the food for AgNO3 and AgNP treatments, respectively. These values decreased by 80 % and 93 % within 48h depuration in el-soil, for AgNO3 and AgNPs, respectively. After two months depuration, 97 % and 99 % of the accumulated Ag from Ag ions and AgNPs, respectively, were excreted. Bioaccessible Ag was defined as the sum of Ag extracted by water and ammonium acetate. A rapid reduction of Ag ions occurred when they get in contact with soil organic matter, turning Ag ions into NPs and colloids. Soil properties had a limited impact on Ag speciation. Interestingly, the bioaccessible fraction increased over time in case of AgNPs, contrary to what happened with AgNO3. We showed that despite the very low concentration of AgNPs in soils, AgNPs were bioavailable. After two months, only very little is known about how metal nanoparticles behave once released into the environment and even less is know about the effect of these particles on terrestrial organisms.

The purpose of this investigation was to characterize the difference in toxicity and the impact of ageing between silver nanoparticles and silver nitrate in OECD soil on the earthworm Eisenia fetida. The potential to modify the toxicity of a silver nanoparticle contaminated soil over time is an important temporal consideration when conducting a terrestrial risk assessment.

A set of Earthworms were exposed to the AgNO3 and AgNP spiked soils 24 hours after initial preparation and then a second set of earthworms were exposed to the soils aged 28 days. Both exposures lasted 14 days with endpoints of mortality and body burden. OECD soil treatments were prepared with dissolved AgNO3 or suspensions of 30-50 nm Ag nanoparticles (NP). Soil treatments were split in two and one batch was allowed to age for 28 days in an incubator and kept at constant moisture. The aged soils were sampled every 7 days and subjected to a sequential extraction procedure to evaluate changes in Ag speciation.

Results from the first exposure showed high mortality for worms exposed to silver nitrate and almost no mortality for those individuals exposed to Ag nanoparticles. The exposure to soils aged 28 days showed a decrease in mortality in individuals exposed to the AgNO3 treatments and an increase in mortality in those exposed to the aged AgNP treatments. The AgNP batch was split for both AgNP exposures were indistinguishable while there was a decrease in the Ag body burden for earthworms in the AgNO3 exposures aged 28 days as compared to freshly amended soils. There were changes is the speciation of Ag over the 28 day ageing period in both soil treatments that point toward an increase in the potentially bioavailable pool of metal in AgNP amended soils as compared to the AgNO3 amended soils. Based on these results, the potential exists that morphological changes to the AgNPs in the soil matrix occurred during ageing and that these changes in conjunction with more bioavailable Ag made these treatments more toxic to exposed individuals.

The purpose of this investigation was to characterize the difference in toxicity and the impact of ageing between silver nanoparticles and silver nitrate in OECD soil on the earthworm Eisenia fetida. The potential to modify the toxicity of a silver nanoparticle contaminated soil over time is an important temporal consideration when conducting a terrestrial risk assessment.

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Results from the first exposure showed high mortality for worms exposed to silver nitrate and almost no mortality for those individuals exposed to Ag nanoparticles. The exposure to soils aged 28 days showed a decrease in mortality in individuals exposed to the AgNO3 treatments and an increase in mortality in those exposed to the aged AgNP treatments. The AgNP batch was split for both AgNP exposures were indistinguishable while there was a decrease in the Ag body burden for earthworms in the AgNO3 exposures aged 28 days as compared to freshly amended soils. There were changes is the speciation of Ag over the 28 day ageing period in both soil treatments that point toward an increase in the potentially bioavailable pool of metal in AgNP amended soils as compared to the AgNO3 amended soils. Based on these results, the potential exists that morphological changes to the AgNPs in the soil matrix occurred during ageing and that these changes in conjunction with more bioavailable Ag made these treatments more toxic to exposed individuals.

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of these compounds in the period of 2005-2009, a multiple linear regression was applied to the concentrations of these chemicals in all three phases combined together. PBDE concentrations showed decreasing trends over time, with halving times of ~6 years. The concentrations of HBB and BTBPE are decreasing with halving times of ~10 years. PBEB and DBDPE concentrations did not show any change between 2005 and 2009, suggesting continuing source for these compounds.

**ECO5A-4**

Assessment of persistent organic pollutant in the atmosphere of Latin America

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The widespread use and distribution of pesticides, industrial and urban chemicals and the consequent release into the environment, is of great worldwide relevance. Atmospheric transport is responsible for pollutant dispersion over long distances. As part of an atmospheric regional network, a monitoring program involving the use of air and soil samples was conducted. Pesticides were used to evaluate latitudinal and longitudinal transport processes in Latin America and Patagonian regions from Argentina and Chile. The objective of this work is to revisit the modelling of soil-air exchange by fitting this model to the most extensive database available for concurrent measurements of soil concentrations, fugacities in soil and fugacities in ambient air. The application and recommendation for modelling strategies will be provided dependent on the occurrence of currently used pesticides found, particularly associated with agricultural areas, how DDE, the DDT metabolite, is also found in all sites independently of particular sources, as a consequence of their extensive past use in the region. Regarding PCBs, a predominance of #110, #113, #138, #153 and #209 is related with punctual sources. Considering the latitudinal gradient, until the southeastern region of Argentina, an increasing in DDE, PCBs and PBDEs (BDE-28 and 47) is observed inferring an atmospheric transport of POPs and increased PBDEs and PCBs levels in T. bergeri. Find the close correlation in the sentinel with a clear hot spot near a disposal site, that is accomplished by the presence of PBDEs 28, 47, 99 and 100 and PCBs 138, 118 and 110. In all industrial or urban sites the relation PBDEs/PCBs > 1 reflect the general trend of diminishing PCBs levels and increasing the emergent PBDEs.

**ECO5A-5**

Occurrence of currently used pesticides and selected degradation products in agricultural regions of Western Canada

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Initial studies on occurrence of currently used pesticides in agricultural regions of Western Canada focused on the Lower Fraser Valley where berries and fruits are the dominant crops, and the Canadian prairies where grains and oil seed crops dominate. These two agricultural regions were selected due to their differences in crop types, climate, and expected use of pesticides. Western Canadian agricultural regions have the highest usage of pesticide use in Canada. In the initial studies we focused on key pesticides that we suspected to be of concern and prior to the availability of a 2003 usage inventory. These pesticides had little information about their atmospheric occurrence, methods of occurrence, and the potential for atmospheric transport. The recent development and application of a soil fugacity sampler allows for the first time to validate unequivocally the general trend of diminishing PCBs levels and increasing the emergent PBDEs.

**ECO5A-6**

Semi-volatile organic pollutants and trace metals associated with Saharan dust air masses: estimated inhalation exposures at source and downwind sites

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Saharan dust air mass transport eroded mineral dust, chemical contaminants, and microorganisms in the atmosphere thousands of kilometers from the Sahara/Sahel of Africa to the Americas, Europe and Asia. Global ocean-atmosphere interactions, regional meteorology, surface material composition, and human activities are the primary factors driving the composition and quantities of transported dust particles and associated contaminants. Semi-volatile organic compounds (SOCs) such as banned and current use pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) have been detected in dust air masses over source (Mali) and downwind (California, USA, Canada, Chile, and Argentina) regions. The predominant factors driving the composition and quantities of transported dust particles and associated contaminants. These processes also play a major role in the atmospheric transport and detection of these contaminants in the atmosphere in the recent to a longer time frame.

**ECO5B-1**

Re-visiting the modelling of soil-air partitioning, fugacities in soils, and soil-air exchange of persistent organic pollutants

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Soils are the main reservoir of persistent organic pollutants in the environment and air-soil exchange of POPs is a key process affecting the atmospheric occurrence of POPs and the extent of soil as a reservoir of pollutants. The direction of the air-soil exchange can be determined by comparing the POP fugacity in soil (fs) and the fugacity in ambient air (fa). If fs is higher than fa, then there is a net volatilization, while if fs is lower than fa then there is a net deposition. If is usually estimated from the soil-air partition coefficient (KSA), which is often estimated from the octanol water partition coefficient. Lately, polycationic linear free energy modifiers (pp-LFER) have also become popular for modelling the environmental partitioning of POPs. The development and application of a soil fugacity sampler allows for the first time to validate unequivocally these models. The objective of this work is to re-visit the modelling of soil-air exchange by fitting this model to the most extensive database available for concurrent measurements of soil concentrations, fugacities in soil and fugacities in ambient air. The application and recommendation for modelling strategies will be provided dependent on the chemical properties, environmental parameters such as weathering and soil characteristics. Implications for modelling soil-air exchange will also be given.

**ECO5B-2**

Provision for estimating atmospheric lifetimes of micropollutants using the Junge relationship

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The Junge relationship between lifetime and atmospheric concentration is a consequence of incomplete mixing of gases in the global atmosphere. At the limit, with gases very long lifetimes approach a homogeneous distribution in the earth's atmosphere. More generally, variability in atmospheric concentrations of gases with shorter lifetimes measured at remote locations will be greater than variability of gases with longer lifetimes. Results presented in this pilot study that aims to take advantage of the ubiquitous presence of cyclic volatile methylsiloxanes (cVMS) in the global atmosphere to calibrate the Junge relationship and estimate atmospheric lifetimes for other pollutants. The cVMS have many advantages...
as benchmark substances to calibrate the Junge relationship; namely; 1) They are high-production volume chemicals with high release rates and high concentrations in the atmosphere; 2) Their atmospheric lifetimes are well-understood based on a combination of laboratory and field studies that have been verified with mass balance modeling; and 3) They are emitted from urban areas, which coincides with source areas for many other atmospheric micropollutants of interest and opens the possibility of extrapolating Junge relationships for the cVMS to estimate atmospheric lifetimes of other atmospheric micropollutants. We have analyzed two datasets for evidence of variability consistent with the Junge relationship: Measurements at 12 sites of the Global Atmospheric Passive Sampling (GAPS) network, and measurements made at a rural site in southern Sweden, and found strong Junge relationships in both datasets. We view these relationships as proof-of-concept that the cVMS can be used to calibrate the Junge relationship and enable quantitative estimation of atmospheric lifetimes of organic micropollutants if they are measured simultaneously and have similar source profiles and sink processes. Measurement of silicones and other selected pollutants in air at the full complement of over 50 GAPS sites is currently underway, and will provide a more extensive data set for testing the Junge relationships in future work. Particularly intriguing is the potential for applying the relationship to estimate atmospheric lifetimes of substances with significant sinks from dry and wet deposition.

EC05B-3

On the contribution of biomass burning to POPs in air in Africa

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Forest, savannah and agricultural debris fires in the tropics and subtropics are sources for widespread pollution and release many organic substances into air and soil, including persistent organic pollutants, i.e., polychlorinated dibenzo-p-dioxins and -furans (PCDD/Fs) and polycyclic aromatic hydrocarbons (PAHs). The significance of this source for the exposure of humans and the environment is unknown.

The global multicompartment chemistry-transport model MPI-MCTM [1] is used to predict atmospheric concentrations of selected PCDDs and PAHs. The model-scale meteorology was constrained by nudging the atmospheric sub-model to re-analysis data. Global emissions of PAHs and PCDDs into air are based on recommended (PCDDs) and selected (PAHs) emission factors applied to fire distributions. Daily real-time fire data are based on satellite-observed fire radiative power measured from satellite (MODIS instrument) [2]. No other primary sources are considered. Model-predicted near-ground concentrations of PCDDs and PAHs are compared with observations during January-June 2008 at a number of stations across Africa [3]. Back-trajectory analyses suggest that some of these may have been influenced by fire episodes in Europe.

Continental half-year (Jan-June 2008) mean near-ground atmospheric concentrations are 0.0076, 0.51 and 3.25 fg m-3 of 2,3,7,8-TCDD, 1,2,3,4,6,7,8-HpCDD and OCDD, respectively. It is found that open fires can explain a major fraction of the air pollution by PCDDs in the background of west, central and southern Africa. Highest concentrations of PCDDs and PAH are predicted in 1-4 km altitude throughout most of the time, sometimes even higher and sometimes near the ground.

tested in PCB contaminated sediments, which do not have acute toxic effect on the test organisms. The measured parameters included PCB bioaccumulation, feeding activity, biochar (charcoal) and compost for their ability to reduce the desorption and biodegradation of phenanthrene as a model PAH in three different sandy loam soils (Outfield, RS, or Olsen) and to reduced toxicity, but on the other hand this might also decrease biodegradation. The aim of this study was to characterize the soil amendments activated charcoal (AC), in control sediments the worms were gaining weight related response in all of the studied ecological parameters. The ecological responses were also sediment dependent. The AC also reduced the biomass of the worms compared to the control; moreover in two sediments out of three the AC addition in the sediment ultimately led the worms to lose weight during the experiment period, whereas in control sediments the worms were gaining weight. The effects of biochar on the biological responses were smaller than that of the AC. The site-specific evaluation is particularly important when remediation measures are designed. Negative effects of carbon amendments to the organisms, such as change in behavior, reduced growth and reduced reproduction needs to be considered, since they are important factors affecting the assessment of toxicity, bioaccumulation and ecological quality of the sediments.

Effects of carbon amendments on ecological responses and PCB bioaccumulation in Lumbriculus variegatus

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During the recent years synthetic black carbon products (mainly activated carbons AC, but also biochars), have been under an intense study to be used as a potential stabilization method for contaminated sediments. Results from various studies both in laboratory and pilot scale field studies have supported the high efficiency of AC amendments in sorption, and consequential reduction of bioavailability of HOCs. However, recent studies have shown that AC amendments may inflict adverse ecological effects on aquatic organisms, e.g. sediment avoidance, inhibition in growth and alteration in sediment ingestion. The direct biological effect of AC on organisms may have an influence for both laboratory-scale bioaccumulation and toxicity testing and field-scale ecological impact assessment. The aim of this study was to test the responses of carbon amendments to sediments on oligochaete Lumbriculus variegatus in three sandy soil sites (Outfield, RS, or Olsen) and for determining a solution and can be used to break the infinite circle of new site investigations.

Monitoring over several years at pilot-scale application sites at Hunters Point, CA and Grasse River, NY USA has demonstrated that AC amendment reduces contaminant availability with biodegradation when comparing live and sterile soils. The strongest effect of biodegradation on PAH availability was found in unamended soil, with 75% difference between sterile and live soil slurries, while the lowest PAH availability was measured in PAC amended, live soil. The combination of the chemical and microbial studies suggested that microorganisms with the ability to degrade PAHs persist long-term in soils, regardless of the presence of activated carbon amendments which reduce the PAH availability, presumably because they utilize other soil organic matter as their main carbon source.
to reduce pore water concentration at Hunters Point was compared to measured values for up to 5 years of field aging. Insights drawn from this modeling effort help to explain results from the sorption study. Values of $K_{AC}$ for field-aged AC were lower than freshly-added AC for spiked PCBs up to a factor of 10, while the effect was less for native PCVs. For both Hunters Point and Grasse River field-aged AC, similarly diminished sorption compared to fresh AC for the spiked PCbs was observed. However, there was a decrease in sorption coefficients compared to values for fresh AC for most of the native PCBs in the Grasse River sediment which is likely due to the nearly ten times higher organic carbon content. Mass transfer modeling for Hunters Point indicates that the mass transfer limitations of PCB sorption may change over time in relation to natural organic matter (NOM). The AC does not lose sorption capacity, yet incremental improvements may slow with time. Site characteristics such as the contaminant desorption kinetics and NOM quality and quantity will be important considerations when designing sustainable remediation measures with AC amendment. Importantly, these studies show that aged amended AC continues to effectively sorb PCBs several years following field application.

EC06A-6
Impact of biochar on the biodegradation and bioavailability of organic contaminants in soil
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The fate of hydroporphic organic contaminants (HOCs) is often controlled by the organic matter content of the soil. However, organic matter may further be mineralised or degraded by microorganisms and eventually release such organic contaminants. In order to enhance remediation of HOCs, biochar, which is a recalcitrant carbon rich product from the pyrolysis of biomass can be utilised. This study investigated the influence of 0%, 0.1%, 0.5% and 1.0% of different wood waste biochar (BC1 and BC2), and the influence of 0%, 1%, 5% and 10% of BC1 (2 mm and 3-7 mm) on the relationship between microbial mineralisation and hydroxypyrrol-β-cyclodextrin (HPCD), CaCl2 and CaSO4 in bituminous coal. The amendment conditions were (1): 0% and azoxyestradiol in sand (0.35), 0, 30, 70 and 140 days (azoxystrobin), and 0 and 40 days (phenanthrene). The total extent of $^{14}C$-associated mineralisation was assessed by monitoring $^{14}C$-associated mineralisation over 14 days in respired isotope assays and compared to HPCD, CaCl2, and methanol extractions. Results showed that BC1 and BC2 amendments showed significant reduction ($p < 0.01$) in extent of $^{14}C$-phenanthrene mineralisation and extraction compared to 0%. However, the reduction was greater in 2 mm BC1. There was little or no mineralisation of $^{14}C$-azoxyestradiol but BC1 and BC2 showed reduction in extractability. Linear correlation between HPCD extractability and total amount mineralised ($^{14}C$-naphthalene and phenanthrene) revealed very good correlation in all concentrations of biochar amendments BC1 ($r^2 = 0.94$, slope = 0.94, intercept = 0.86) and BC2 ($r^2 = 0.94$, slope = 0.90, intercept = -1.34) for naphthalene and phenanthrene. Additionally, the CaCl2 and methanol extractions underestimated and overestimated extent of mineralisation, respectively. This paper thus suggests that biochar used in this study can reduce the bioaccessibility of HOCs and that HPCD extraction strongly predicts the bioaccessibility of naphthalene and phenanthrene in soils amended with biochar. Thus, biochar may be a useful tool in sorption of pesticides to prevent leaching to ground water. However, the production process, particle size, feedstock and soil properties determine the capability of biochar to adsorb organic contaminants.

EC06B-1
Toxicity and bioavailability of geogenic polycyclic aromatic compounds from coal
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Fate of geogenic polycyclic aromatic hydrocarbons (PAHs) with high molecular weight present in coal or coal products is often studied. The main objective of these studies is to evaluate the toxicity of PAH and their metabolites. New findings in this area concern the toxic potency of PAHs in aquatic systems. From a long-time exposure of aquatic organisms to PAHs, genotoxicity and carcinogenic effects are observed. Abiotic processes like adsorption and photochemical degradation also influence the bioavailability of PAHs in aquatic systems. In this study, we evaluated the genotoxicity of PAHs in natural aquatic systems by using the marine fish Danio rerio. Both in vitro and in vivo genotoxicity assays were performed. PAHs and their metabolites were detected in the body fluids of fish and their toxicity was compared to standard chemicals. The results showed that PAHs can cause genotoxicity in aquatic organisms and their bioavailability can be influenced by abiotic processes. This study provides new insights into the toxicity of PAHs in aquatic systems.

EC06B-2
Adsorption of organic contaminant from aqueous solution on natural porous material
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The study investigated the adsorption of organic contaminants from aqueous solution on natural porous material. The adsorption experiments were conducted with different types of adsorbents, including activated carbon, zeolites, and clay minerals. The adsorption capacity of these materials was evaluated using a variety of techniques, including equilibrium measurements, kinetic studies, and thermodynamic analysis. The results showed that the type of adsorbent and the nature of the contaminant significantly affect the adsorption process. The study also investigated the influence of various factors, such as pH, temperature, and ionic strength, on the adsorption of organic contaminants. The findings of this study provide valuable information for the design and optimization of remediation strategies for contaminated environments.

EC06B-3
Novel pathways in the adsorption of weak organic acids by black carbon leading to ionization constant shifts on the surface
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The adsorption of weak organic acids (WOAs) by black carbon (BC) is a complex process that involves both physical and chemical interactions. WOAs are typically characterized by low pH and high acidity, and they are highly soluble in water. The adsorption of WOAs by BC can lead to the formation of surface complexes, which can alter the ionization constant of the WOAs. In this study, we investigated the adsorption of WOAs on BC using a combination of experimental and theoretical approaches. We found that the adsorption of WOAs on BC can lead to significant changes in the ionization constants of the WOAs. These changes are the result of electrostatic interactions between the WOAs and the BC surface, and they can be used to improve the understanding of the behavior of WOAs in the environment.
surface (termed ps−/ps+EDA). At intermediate pH values, the ps−/ps+EDA bond drives a positive shift in the pKₐ of SMT and an increase in the stability constant for the zwiterion SMT+/− on the surface relative to the respective value in water.

We conclude that adsorption of weak organic acids on black carbon surfaces can result in significant negative impact on ionization constants on the surface driven by the formation of strong complexes, such as low barrier H bonds and ps−/ps+EDA interactions.

EC06B-4 Determining sorption of cationic surfactant to organic matters and clay minerals: An application of Ion-Exchange SPME method

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Sorption property is crucial for the mobility of organic compounds in environment and hence is highly relevant to the bioavailability of those chemicals. The freely dissolved is mostly regarded as the bioavailable fraction and often measured by passive sampling techniques. However, sorption coefficients have been demonstrated in both bioavailability and bioaccumulation processes. In this study, a 7-μm polyacrylate SPME fiber was successfully used as an Ion-Exchange passive sampler for measuring freely dissolved cationic surfactant. Cationic surfactants are permanently charged organics and have high affinity to negatively charged environmental media (e.g. surface of clays and humics). By employing the SPME method, we studied the sorption behavior of benzyl dimethyl dodecyl ammonium chloride (C12-BAC) to different sorbents, including humic acids, clays minerals and an artificial sediment. By identifying the competition between bioavailability and inhibition and measuring the sorption coefficient of the individual sediment component, the sorption behavior to the whole sediment could be estimated, which was comparable to the data for sediment-only sorption experiment. Whether the sorption of cationic surfactant to field sediment could also be extrapolated via such a method requires further research.

EC06B-5 Walking the tightrope of bioavailability: growth dynamics of PAH degraders on vapour-phase PAH

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Effective biodegradation of soil contaminants requires both adequate environmental conditions and a suitable contaminant bioavailability to degrading microbes. Compound bioavailability however, is ‘Janus-faced’: i.e. is essential and likely promoted by the organism for assimilative uptake, whereas, when too high, may lead to toxic effects and provoke avoidance strategies (e.g. tactic responses) of the target organisms. Although the bioaccessible compound pool for both effects may be identical, the exposure of individual organisms to environmental chemicals is governed by their bioavailability and may particularly interfere, where a metabolisable substrate causes toxicity at high bioavailability. Such situation leads to a microbial tightrope walk that is often overlooked in bioremediation studies where one tends to assume that effective pollutant-utilizing bacteria tolerate any exposure to these substrates. The highlights of this paper give evidence of down-gradient, i.e. negative (chemo-)tactic movement of Pseudomonas putida (NAH?) away from a NAH point when exposed to vapour phase NAH (surprisingly at even at gaseous concentrations lower than aqueous concentrations that cause negative chemo-attraction). They secondly elucidate the tightrope walk of substrate bioavailability for assemblage growth and growth inhibition of strain PpG7 in vapour-phase NAPH gradients. Microcosm experiments revealed that high cell densities increased growth rates close (< 2 cm) to the NAH source, whereas intercellular competition for NAH decreased growth rates and biomass at larger distances despite the high NAH gas phase diffusivity. Finally, such varying growth kinetics is explained by a combination of bioavailability restrictions and NAH-based inhibition. To account for this balance, a novel, integrated ‘Best-equation’ describing microbial growth influenced by both bioavailability and inhibition is presented. Our work clearly demonstrates the importance of bacteria to degrade vapour-phase compounds and to influence vapour-phase PAH concentration gradients even at the centimeter-scale. It further underlines the importance of high active biomass and concomitant effective reduction of their exposure to inhibitory substrates in order to create environments favourable for survival, which may influence the exposure dynamics and ecology of entire microbial communities and hence actively shape environments beneficial for enhanced biodegradation.

EC06B-6 Contribution of microbial biomass to non-extractable residue formation from an organic contaminant

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Biodegradation of pesticides in soil is actually understood as their transformation into various metabolites, microbial biomass, mineralisation products and non-extractable residues (NER). NER are believed to mainly consist of hazardous parent compounds or primary metabolites sorbed to or sequestered by soil organic matter (SOM). Up to date, however, their chemical composition remains still unclear. This is because numerous studies on NER formation are limited to quantitative analyses in soils or to simple humic acids-contaminant systems. During biodegradation of organic contaminants, the C is used by microorganisms for their biomass synthesis. After cell death and lysis, biomass components are stabilised in SOM ultimately forming harmless biogenic residues. We investigated the formation of biogenic residues during biodegradation of 13C-labeled dichlorophenoxacyclic acid (2,4-D) in soil over 64 days. We prove, for the first time, that nearly all NER formed from this readily biodegradable herbicide in soil contained only non-hazardous microbial biomass components. Therefore, for the proper assessment of the potential risks of a target contaminant in soil to environment it is necessary to consider a possible biogenic origin of NER in the mass balances of contaminants in soils.

EM01 - Collection and use of monitoring data for environmental risk assessment of chemicals

EM01A-1 Update on the global monitoring plan (GMP) in the UNEP Stockholm Convention on persistent organic pollutants (POPs), technical guidance, data analysis, modelling, assessment and workplan

R.G. Guardians

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The GMP established under Article 16 of the Stockholm Convention on POPs (SC) has been active since 2004 and produced a first global assessment in 2009. It is currently reviewing the technical guidelines, an extensive document describing recommended methods and procedures to monitor POPs in the GMP core media, air and human tissues (milk, serum). The work under the GMP has identified the relevance of modeling long range atmospheric transport including meteorological and climatic variability to understand better the effects of a changing climate on POPs. Important results have been published in the United Nations Economic Commission for Europe’s (UNECE’s) report on Hemispheric Transport of Air Pollution (HTAP) 2010 Part C and the United Nations Environment Programme (UNEP)/Arctic Monitoring and Assessment Programme (AMAP) report of “Climate Change and POPs: Predicting the Impacts”. Challenges for future work include long term stability and coordination of the networks, the analytical and data quality assurance/quality control (QA/QC), data management and archiving, the development and use of models dealing with atmospheric and marine long range transport, as well as modeling ecological and physiological pathways and exposed in leading edge to environmental impacts. This paper describes the main results and identified challenges for future work of the GMP, an international cooperative knowledge building process focused on the fate of POPs released into the environment and the possible ways to decrease or eliminate them.

EM01A-2 Adapting monitoring strategy to the contaminant source characteristic - chromium in the upper Dunajec River watershed

E. Szalinska, J. Dominik

EM01A-3 Factors influencing the quality of river monitoring data used for environmental risk assessment of particulate/hydrophobic chemicals

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EM01A-4 Making management decisions with imperfect data: assessing potential aquatic metal risks with biotic ligand models

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Wca environment, Swindon, United Kingdom

Accounting for imperfect data, through the use of sophisticated Biotic Ligand Models (BLMs), represents the most technically robust method assessing potential metal risks in the freshwater aquatic environment. New user-friendly BLMs (uBLMs) are now available to facilitate their regulatory use. These new models are based on the outputs of the more sophisticated BLMs but require data on fewer water physicochemical parameters to run (i.e. limited to just pH, calcium and dissolved organic carbon). However, there remain obstacles to using the tools, specifically the lack of site-specific physicochemical input data. Absence of required input data means the models cannot be run. Yet, while not always starting with the perfect dataset for all sites of interest, it is rare there is a complete absence of “fit of purpose” input data. There are several ways by which input data gaps can be filled in a robust, precautionary manner to deliver a screening level assessment which can then be used to develop focussed monitoring programmes, identify sensitive sites and broadly characterise risks. This presentation will give an example of how, through the use of imperfect data, management decisions can be made in relation to the assessment of potential aquatic risks of metals.

EM01A-5 Mapping the chemical environment of London: the London Earth project


British Geological Survey, Nottingham, United Kingdom

The British Geological Survey has been involved in a number of programmes to systematically map the concentrations of chemical elements in the surface environment of the United Kingdom. These include both European-wide GeoEcoSurveys’ projects (e.g. FOREGS geochemical atlas and GEMAS metals in agricultural soils) and regional projects in rural and urban areas of England and Scotland. In May 2010 the results for the largest urban mapping survey undertaken, ‘London Earth’, were released. Soils were sampled along a density of 4 km2/km2 from the Greater London Authority. This represents one of the world’s largest systematic geochemical mapping exercises in an urban area. Over 6,400 topsoil samples were determined for more than 50 elements by X-ray fluorescence spectrometry (XRFS) and other soil parameters such as pH and loss on ignition were determined. Since the publication of the results further analyses have been done on subsets of the samples including mercury (Hg) and organic contaminant studies, profile lines mapping the occurrence of Au and PGEs (platinum group elements), and bioaccessibility studies. Applications of the London Earth data to environmental and health issues will be presented.

EM01A-6 Active local sources of PCBs in the Arctic

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Geological Survey of Norway, Trondheim, Norway

In this presentation the work done at Svalbard will be emphasized, this work shows active local sources to PCBs in the Arctic. The levels of PCBs in the primary sources are up to permille levels and the risk of dispersion is evident. These results should be taken into account in the ongoing discussion of local sources versus long-range transport sources from. In this presentation the work done at Svalbard will be emphasized, but also other similar work will be shortly reviewed.

EM01B-1 GEMAS: Geochemical mapping of agricultural and grazing land soils at the European and national scales

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The North American Soils Geochemical Landscapes Project (NASGLP), a collaborative effort among the US Geological Survey (USGS), the Geological Survey of Canada (GSC) and the Mexican Geological Survey, was initiated to provide a soil geochemical database for a broad-based group of users in the field of environment and human health. This Tri-national Survey will ultimately produce a database of the regional natural-occurring differences in concentrations and physicochemical characteristics of soils which can be used to assess background conditions and identify anthropogenic impacts. The survey is based on low-density sampling (within a 40 km by 40 km grid) yielding 13,487 sites across North America. Soil sampling and analysis in Canada was initiated by the GSC in partnership with other provincial and federal agencies including Health Canada and the Canadian Food Inspection Agency (CFIA) for contaminants associated with SPM. Thereby, the sampling frequency or strategy should be carefully chosen in order to obtain information on potential variations of concentrations linked to the river hydrological cycle. Finally, sampling methods should be characterised and compared. In a recent study of the Rhone Sediment Observatory we compared the efficiency of different SPM sampling techniques. A difference in the particle size distribution of SPM sampled with the various techniques was noticed, which could have an effect on the concentrations of contaminants associated with SPM. To conclude, this study revealed that factors such as analytical methods, sampling frequency and sampling methods impact concentrations or annual average concentrations of chemicals in SPM, bed sediment and water, and thus impact the monitoring dataset quality. Consequently, the outcome of an environmental risk assessment based on such a monitoring dataset might be significantly affected. Interlaboratory comparison tests, quality controls, as well as a complete documentation on sampling and analytical methods are among possible solutions to build relevant datasets of chemical concentrations in rivers.
Canada in 2004. Analytical parameters included total metals, pH, total organic carbon, inorganic carbon and loss on ignition. A sub-set of the samples collected in Canada from the surface (0-5 cm, referred to as the public health layer) and the C horizon were analyzed for metal bioaccessibility using a simplified physiologically based extraction test as a surrogate for bioavailability. Results will be presented on the bioaccessibility of naturally-occurring metals in the soil public health layer and the C horizon, along with relationships of bioaccessibility with soil geochemical data. The data obtained indicated some differences in mean elemental bioaccessibility between the public health layer (0 - 0.5 cm) and the C horizon (parent material) for each province. There were also some statistically significant relationships between metals bioaccessibility and soil pH, total organic carbon and loss on ignition for a sub-set of the samples. Comparison of the elemental bioaccessibility in the natural background soils to literature information on bioaccessibility from contaminated sites (e.g., mine sites) in Canada will also be shown, along with how the data might influence derivation of generic soil quality guidelines in Canada.

EM01B-3 Quality assurance in the GEMAS project and results of the connected proficiency test

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The comparison of analytical results from aqua regia and acid digestion of soil samples for the GEMAS project was performed in 2011 to check the comparability of individual national laboratory data with the data used for mapping, and to confirm the trueness of the mapping data. In total 21 institutions from 16 countries submitted analytical data, including the mean values from QC analyses as “normal participants”. Evaluation of data and laboratory work was done using robust statistics, limited standard deviations and z scores. Results are presented for between-lab-reproductibilities, Horwitz ratios as indicator for PT performance, and inter-method discrepancies between analytical methods and sample preparation techniques. The assessment of the QC data for the standards AP and Gr in this PT confirms the trueness of total element contents and most AR contents.

EM01B-4 Comparison of XRF and Aqua Regia data from agricultural soil in Europe: results from the GEMAS project

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The use of these GEMAS monitoring data for regional and local risk assessments of metals in soil will be discussed based on examples for several metals.
Environmental chemistry and toxicology face the immense challenge of protecting thousands of species from thousands of substances released into thousands of different landscapes. These challenges are limited because of financial, practical, ethical and time-space restrictions. To cover all relevant cases, fate and exposure models have been developed. Yet, such models too, tend to become data hungry because of parameterization. Properties of chemicals have extensively been used to extrapolate knowledge, allowing one to estimate default values for parameters in the absence of experimental data. The objective of the present paper is to extend this approach to species traits and landscape characteristic. In particular, we aim to identify similarities and differences in size scaling in the various disciplines involved, i.e. hydrology, chemistry, biology and technology.

Confining ourselves to lakes and organisms, we will show similarities and differences in the turnover of water, energy and chemicals. Both data and theory suggest that inflow in lakes geometrically while inflow in organisms (ventilation, consumption etc.) increases allometrically. Understanding the overall principles determining the flow of water and biomass helps us to predict the fate and accumulation more accurately. Rather than choosing a "typical" value for run-off in fate models, we might choose the average and standard deviations noted for catchments of a certain size. Instead of picking an uptake rate constant for accumulation from a more or less related species, we can now estimate the kinetics in poorly investigated specimens from their body size. In addition, overall principles as size scaling open up opportunities to design generic models.

While we focus on the approach for size scaling, other variables can help to refine explanations. Estimations can be improved by adding temperature as an explanatory variable, allowing one to distinguish between temperate and tropical regions or between cold-blooded and warm-blooded animals. In addition, size scaling can also be applied to other parts of the environmental cause-effect chain. As an example for emissions of carbon dioxide and polyyclic aromatic hydrocarbon, fuel use by engines scaled similarly to running organisms. At the same time turnover and species abundance (size spectra) in ecosystems can be related to environmental stressors.

EM02A-3
Not PBT, but LRTP - Identification of substances with possible very high concern
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In the European chemicals legislation REACH, a substance is considered of very high concern if it is persistent, bioaccumulative and toxic (PBT). A substance’s long-range transport potential (LRTP) and the subsequent hazard to remote regions are not explicitly included but can give cause for serious concern. To identify compounds, which would not be classified as PBT substances but are prone to LRTP (non-PBT-L substances), we screened the Canadian Domestic Substance List (CDSL), which contains 22,438 compounds in use on the Canadian market.

In a first step, the CDSL was searched for organic compounds which are to more than 95% in the neutral form at an environmentally relevant pH range. The identified compounds were run through EPI Suite v4.0 such that substance properties were quantified by the implemented estimation methods. Next, the list was edited in two ways to identify compounds with LRTP. On the one hand, the half-life on the 100 days or longer range transport (LRTP) of substances was cut off (half-life in air > 2 days). On the other hand, all compounds were simulated with the multimedia model ELPOS v2, which calculates relative indicators for persistence and LRTP (Matthews et al. 2009). Applying the half-life criterion of 2 days in air as an indicator for LRTP, we identified 621 substances, which are prone to LRTP; 594 of these are not PBT. In contrast, investigations with ELPOS lead to a shorter list of 188 substances which are not PBT but show LRTP (non-PBT-L substances). All 188 non-PBT-L compounds are persistent, but not bioaccumulative. However, in order to decide if these substances are of very high concern, the list was compared with both potential Arctic contaminants identified by Muir and Howard (2006) and by Brown and Wania (2008), respectively. This combination of different screening methods allows to focus on a subset of eleven organic substances, which are not PBT but identified to be characterized by LRTP. An additional literature survey about substance properties elucidates possible hints on the reliability of the screening procedure.

References

EM02A-4
New read-across model to estimate the BCF for fish from similar chemicals
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The bioconcentration factor (BCF) is a key property to assess the bioaccumulative properties of organic chemicals. Existing estimation models relate the BCF for fish to the octanol/ water partition coefficient. Available experimental data from literature have been collected and thoroughly validated, resulting in a high quality data set of BCF for fish covering 1000 organic chemicals. The read-across approach calculates the BCF for similar compounds by a similarity weighted averaging. The selection of the similar compounds and the numerical calculation of the similarity weights are achieved by atom centred fragments (ACF).

The similarity threshold to consider reference compounds can be fine-tuned to balance accuracy and generality. With the default threshold, the model yields valid results concerning the trends among the chemicals. The statistical certainty of the calculated reliability of the model can be increased by inspecting the value diversity and the molecule size ranges for the selected compounds. A relationship between the estimation errors and these ranges can be shown. The model performance roughly compares to the literature models. The errors of the new approach are not correlated individually per compound to the respective errors of the other models. However, it can be shown that generally agreement of the prediction results is related to small estimation errors. This offers the opportunity to apply a suit of models for consensus modelling.

The new model as well as the studied literature models are already implemented in the software system ChemProp. The software offers uncertainty estimation for the result in addition. ChemProp is publicly available for free, based on a bilateral license agreement.

The development of this method has been supported by the EU Integrated Project OOSIS (Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Test Information, contract No. 037017). A part of the data was compiled within the former EU project CAESAR (Computer Assisted Evaluation of industrial chemical Substances According to Regulations, contract No. 022674).

EM02A-5
Modelling the impact of dietary transitions on human exposure to bioaccumulating organic contaminants
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By combining models of a contaminant’s fate in the physical environment with models of bioaccumulation through the food chain, it is now possible to describe mechanistically and dynamically the entire sequence of events linking emissions and body burden in humans. Here we rely on such a model combination to examine the role of dietary transitions on human contaminant levels. Concentrations of persistent organic pollutants (POPs) in Inuit populations have been observed to decrease over the last decade or so. Are these decreases an indication of the success of international efforts to reduce the emissions of POPs globally or did they occur because the diet of the Inuit has shifted away from traditional food items toward store-bought food? It is assumed that the diet of the Inuit is composed of regionally harvested traditional food and of food imported from the South. The global contaminant fate model is used to calculate time trends of contamination in the physical environment of the Arctic and the Northern temperate zone over the past 100 years. Using the calculated contamination time trends in the Arctic environment as input, the contamination of the traditional food chain and of the imported food is calculated using the ACC-Human bioaccumulation model. The contamination of the imported food is similarly calculated using ACC-human and the calculated contamination time trend in the temperate environment. Longitudinal body burden age trends of a number of individuals representing different sections of Inuit society are simulated: Those maintaining a predominantly traditional diet throughout their lifetime, and others who underwent dietary transitions at different times, ages, and to a different extent. By combining multiple longitudinal body burden age trends calculated for individuals born in different years, cross-sectional body burden age trends can be generated. These allow one to compare those obtained with those based on cross-sectional human biomonitoring studies. The model predicted concentrations of POPs decline over the past decade both in Individuals who maintained a traditional diet as well as those that increasingly relied on store-bought food from the South for sustenance. The latter group is predicted to have experienced a faster decrease in exposure. Transitions in the diet from traditional to store-bought food may contribute at least as much to declines in contaminant concentrations in Inuit as declines in environmental contaminant concentrations.

EM02A-6
Modelling approach to estimating the source strength of cyclic volatile methylsiloxanes from the city of Zurich, Switzerland
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The global contaminant fate model is used to calculate time trends of contamination in the physical environment of the Arctic and the Northern temperate zone over the past 100 years. Using the calculated contamination time trends in the Arctic environment as input, the contamination of the traditional food chain and of the imported food is calculated using the ACC-Human bioaccumulation model. The contamination of the imported food is similarly calculated using ACC-human and the calculated contamination time trend in the temperate environment. Longitudinal body burden age trends of a number of individuals representing different sections of Inuit society are simulated: Those maintaining a predominantly traditional diet throughout their life-time, and others who underwent dietary transitions at different times, ages, and to a different extent. By combining multiple longitudinal body burden age trends calculated for individuals born in different years, cross-sectional body burden age trends can be generated. These allow one to compare those obtained with those based on cross-sectional human biomonitoring studies. The model predicted concentrations of POPs decline over the past decade both in Individuals who maintained a traditional diet as well as those that increasingly relied on store-bought food from the South for sustenance. The latter group is predicted to have experienced a faster decrease in exposure. Transitions in the diet from traditional to store-bought food may contribute at least as much to declines in contaminant concentrations in Inuit as declines in environmental contaminant concentrations.
The cycle volatile methylsiloxanes (cVMS), octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6), are widely used in personal care products. Given their high volatility resulting in a large fraction of the used amounts being emitted to the atmosphere, their persistence in the environment, and their large capacity to change transport, it is important to improve knowledge of their sources to provide a basis for future environmental fate modelling and air assessment and management of cVMS. In this study, we present a novel approach to estimating the urban source strength of cVMS by employing a multimedia mass balance model in combination with measured cVMS concentrations in air, and apply it to D4, D5 and D6. Difference variations of ambient air concentrations of D4, D5 and D6 were measured at two sites in Zurich, Switzerland, in winter 2011. One site was located in the valley near the city center of Zurich which was chosen for the same reasons as the site on the Rhine river was chosen to understand the influence of urban emissions on the partitioning of cVMS in the boundary layer. A multimedia mass balance model was developed and employed to (i) calculate the concentration trends observed at both sites in terms of dominant processes and (ii) estimate urban source strengths. Choosing the emission rate as an adjustable model parameter made it possible to estimate the source strength based on empirical data gathered during the study or available in literature. Key parameters are the measured cVMS concentrations, inversion layer height, temperature, wind speed, and chemical substance properties of D4, D5 and D6. Levels of D4, D5 and D6 in air in the city center were 14.1, 64.3, and 57.1 ng/m³, respectively. Concentrations on the hill were lower (3.8, 22.0, 14.5 ng/m³). There was an increase in the concentrations by about a factor of three in the city during our sampling period, and this could be attributed to the model to the enrichment of the chemicals in the boundary layer. Boundary layer height, which varied considerably throughout the sampling campaign, was determined to be the dominating factor. Degradation by OH radicals and deposition were of less importance. For D3, our estimated source strength compares well with estimated emission rates derived from studies on emission rates from individual buildings or emissions estimates based on market information.

EM02B-1 Scenario-study of the expected cumulative impact of European Union environmental legislation on the aquatic discharges from land - an example with three priority substances D.T. Marinov1, A. Pistochi1, M. Trombetti1, G. Bidoligo1
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In order to support the implementation of the Marine Strategy Framework Directive, it was carried out an evaluation of the impact of EU environmental legislation on the quality of the marine environment, with reference to POPs, to support the management of the areas. The work described outcome of scenario analyses up to 2020 for Lindane (gamma-HCH), Trifluralin and Perfluorooctane sulfonate (PFOS) taken as pilot substances. We assessed the exposure levels and the time needed to reach national average disaggregated concentrations for 2016 and 2020. Based on a source term scenario, the future variation in three climate change parameters, temperature, wind speed and precipitation, affect the environmental fate and distribution of POPs in the Baltic Sea region using the POPCATCHING model. The model was applied to the entire range of possible perfectly persistent POPs within the following property ranges; 0 < log Kow < 12.5, 5 < log Kow < 14, and 6 < log Kow < 15. Perfect persistence was assumed for all the POPs to represent a boundary scenario in which the effects of redistribution of POPs due to a changing climate are maximized. Two greenhouse gas emission scenarios were adopted which are referred to as A2 and B2 representing high and low CO2 emissions, respectively. A baseline ("present scenario") was adopted as a control scenario for comparison with the future climate change scenarios. Quotients were calculated between predicted POP concentrations in air based on the A2 or B2 climate change scenarios versus the baseline scenario. The results show that the influence of climate change on the concentrations of POPs in air is strongly dependent on the partitioning properties and emission characteristics of the substances, and that although climate change-induced effects on partitioning of POPs may work to counteract emission reductions efforts for some POPs, in other cases climate change may hasten the removal of POPs from the atmosphere.

EM02B-2 Modelling assessment of climate change-induced effects on the fate of POPs in the Baltic Sea region K. Deguo, C. Matthew, C.T. Ian
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The recently released report on the UNEP/AMAP Expert Group stresses that global climate change may cause redistribution of some legacy persistent organic pollutants (POPs) and possibly counteract the efforts which have been made to reduce releases of POPs under the Stockholm Convention. In the present study we assess how predictions of the future variation in three climate change parameters, temperature, wind speed and precipitation, affect the environmental fate and distribution of POPs in the Baltic Sea region using the POPCATCHING model. The model was applied to the entire range of possible perfectly persistent POPs within the following property ranges; 0 < log Kow < 12.5, 5 < log Kow < 14, and 6 < log Kow < 15. Perfect persistence was assumed for all the POPs to represent a boundary scenario in which the effects of redistribution of POPs due to a changing climate are maximized. Two greenhouse gas emission scenarios were adopted which are referred to as A2 and B2 representing high and low CO2 emissions, respectively. A baseline ("present scenario") was adopted as a control scenario for comparison with the future climate change scenarios. Quotients were calculated between predicted POP concentrations in air based on the A2 or B2 climate change scenarios versus the baseline scenario. The results show that the influence of climate change on the concentrations of POPs in air is strongly dependent on the partitioning properties and emission characteristics of the substances, and that although climate change-induced effects on partitioning of POPs may work to counteract emission reductions efforts for some POPs, in other cases climate change may hasten the removal of the POPs from the atmosphere.

EM02B-3 Persistent organic pollutants in the North Sea in the 21st century: simulations with a combined hydrodynamic and fate and transport ocean model K.T. Driscoll1, M. Mathis1, T. Pohlmann1, B. Mayer2
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The fate and cycling of two selected persistent organic pollutants (POPs), PCB 153 and gamma-HCH in the North Sea in the 21st century is modelled with a combined hydrodynamic and Fate and Transport Ocean Model (FANTOM). Large amounts of POPs enter the North Sea system through atmospheric deposition and river inputs, with additional contributions coming from bottom sediments and adjacent seas. To investigate the impact of climate variability on POPs in the North Sea in the 21st century, the scenario model runs for three 10-year periods from the year 2100 are performed. Based on the IPCC A1B and SRES scenario for the 21st century, our hydrodynamic model results show that average water temperature in the North Sea increases by 2 °C, while salinity is reduced by about 0.59 psu. In the atmosphere, temperatures at 2m increase by 2.3 °C. In this way, we attribute differences between the three 10-year simulations to climate change only. A cursory look at the results show that for gamma-HCH the total mass is highest in summer in both water and sediment with a clear seasonal cycle; total mass in water remains steady for each of the 10-year runs but decreases in each of the future runs relative to the first; total mass in sediment increases over time with future runs increasing faster. For PCB 153, total mass in water remains steady while decreasing linearly in sediment; total mass is greatest in water and least in sediment during the winter months when winter storms cause resuspension from the sediment into the water column (this process is also seen in gamma-HCH). Predictions of spatially resolved concentrations of the antibiotic Clarithromycin in the Main river basin (Germany) are presented to demonstrate how the model can support the selection of promising mitigation strategies. A reference scenario was prepared representing the current state of the contamination with Clarithromycin. Two different scenarios were selected to demonstrate the feature of the model for a priori evaluations of the effect of measures: (i) a local scenario in which two of the larger WWTPs discharging directly into river Main were arbitrarily selected to be equipped with an additional ozonation treatment step with 90% removal efficiency for Clarithromycin; and (ii) regional scenario for the eastern part of the main catchment in which we assumed that at least 10% less consumption of Clarithromycin can be
achieved in the area due to a shift in prescription and consumption behavior.

The effect of the scenarios is evaluated by comparison of the concentration profiles of Clarithromycin along River Main. The effect of the technical measure at the WWTP is evident from much lower simulated concentrations. The additional ozonation at another WWTP more downstream only leads to a small decrease in concentrations because ozonation proves to be much less effective in the upstream part, but overall measure provides the same improvement more downstream. From the simulation data at the mouth of river Main into the Rhine, it can be seen that both measures obviously removed only a small fraction of the total emission in the catchment.

This is an effect of the overlying multi-point emissions from the several hundred wastewater treatment plants of which only a small number have been included in the respective measurements. Concluding, the model allows for the identification of local “hot spots” and also an a priori evaluation of potential mitigation strategies. The analysis given shows that for pharmaceuticals sustainable overall reduction of surface water concentrations is hardly to be achieved by local measures even if some of the largest WWTPs are technically refitted.

EMO2B-4
Spatialtemporal exposure assessment of pesticides in flowing waters-results for predicted environmental concentrations in some brooks in Germany
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In 2011 the “Georisk” project of the German Federal Environmental Agency was finalised. Objective of this project was to develop the scientific basis for an integration of more realistic landscape based scenarios into the process of pesticide registration. Here, results of spatialtemporal simulations of PECTWA in flowing water systems are presented. The objective of the simulations was to predict initial environmental concentrations in flowing water bodies resulting from spray drift entries. Based on this the downstream development of these concentrations over space and time with regard to dispersion processes was simulated (PECTWA, Time over Threshold) including different application pattern within two days using a random generator (application time slot 2 days from 8 a.m. to 6 p.m.). We calculated 25 different application scenarios. An adequate GIS-based software-environment and a functional workflow have been developed which make use of high and medium resolution geodata (water bodies, application areas,タイミングvegetation). The observed spatial entity here is a brook in the Hallertau Region, Germany. All hydrological parameters were derived from ground truthing data. Additionally a more generalised approach using artificial scenario-based landscape definitions will also be presented.

While the risk assessment assuming lentic water bodies is based on the comparison of the PECbuts with the RAC, the local exposure pattern predicted by this dynamic model is summarized to the maximum TWA (i.e. 1 h, PECTWA(1h)) and the total duration when the PEC is above the RAC (ToTh). Because the local PEC is depending on the variable timing and magnitude of the pesticide entries upstream, Monte-Carlo distributions provide a set of possible exposure patterns for each segment from which different PECTWA(1h) can be extracted (e.g. minimum, maximum, median). The exposure duration (as ToTh) is based on the calculation of a RACsyn to consider that effect thresholds are higher if the exposure duration is shorter.

The results show a continuous downstream increase of ToTh and a downstream increasing TWA strongly correlated to application patterns and the hydrological parameters. The artificial-scenario-based results show thresholds of hydrological parameters of the flowing water systems where the influence of the hydrological conditions are the most important influencing parameters related to the simulated PECTWA.

EMO2B-6
High resolution GIS maps for predicting the POPs contamination in soil and comparison with measured data
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The assessment of POPs contamination requires reliable spatial maps for burden and flux assessment. In this work, contamination maps were developed and validated at a space resolution of [GREEKX]1 m with a time frame of one day, in an experimental area located in the central Alps, where direct measurements of PCB concentrations in soil and environmental parameters were available for the year 2008.

Physical algorithms calibrated on experimental data were set up for temperature and organic carbon estimation, along the soil profile and across the year, in order to deduce the horizontal, vertical and seasonal distribution of the contamination potential for PCBs in soil (Ksa maps).

The developed maps were validated with an external set of PCB contamination data, giving very good results (e.g. for CB-153, R2 = 0.80, p-value ≤ 2.2·10-06). The obtained regression coefficients were used for the mapping of the actual soil contamination (concentration maps), taking into account the temporal shifts in soil concentrations from the measurements (as defined by Ksa values). These maps offer the opportunity to evaluate burden and fluxes with highly resolved temporal and spatial detail, and therefore with a high degree of ecological realism (emission maps).

EMO2C-1
Comparison of far field and near field exposure from consumer products for chemical exposure prioritization
D.S. Li, S. Chung, A.D. Henderson, O.J. Jollét
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Traditionally fate and exposure modelling for Risk Assessment and Life Cycle Assessment of products has been focusing its effort on multimedia modeling of far field exposure. However, direct exposure of consumer products via either indoor air (e.g. for indoor furniture or floor) or via direct dermal contact (e.g. for cosmetics or toys) may represent a dominant exposure. The present project therefore aims at developing metrics and method to consistently compare near field and far field exposure to chemicals in products and at testing them on a serie of case studies of three consumer products.

Direct releases from consumer products are characterized by a new metric - the Product Intake Fraction - that determines the fraction of a chemical in a product that is taken up by humans during its use and disposal phase.

Measured releases for a pacifier plasticizer used in typical average conditions leads to relatively high product intake fraction of 6-10-2 and modeled product intake fraction for different shampoo ingredients are in the range of 10-3 to 10-1 depending on the considered chemical properties. For indoor releases of the flooring material, indoor intake fraction is high in the range of 10-4 to 10-2.

Comparison between impacts of direct consumer exposure to those linked to far field life cycle emissions shows that direct impacts are of the same magnitude as or higher than indirect life cycle impacts. Similarly the impacts of indoor releases of a flooring material are of the same order of magnitude as the respiratory effects of outdoor emissions. An uncertainty analysis shows that that the extrapolation from acute to chronic toxicity was the major source of uncertainty (54.1%) and that the three main substances contributing to the impacts also dominated the uncertainty with 94.6% of the total uncertainty.

EMO2C-2
Assessment of local impacts of municipal solid waste management using life cycle assessment (LCA)
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This research takes part in a PhD work whose main aim is to develop a methodology to assess locally the environmental impacts of municipal waste management (MSW) in order to provide environmental elements to local decision-makers and stakeholders.

MSW is a local issue managed under the responsibility of local authorities. To integrate environmental considerations, decision-makers often use Life Cycle Assessment (LCA).

However, the LCA methodology does not take into account the characteristics of the territorial involved and consequently not allow local assessment. Nevertheless, such a consideration appears necessary for local issues such as toxicity and odours. To solve the problem of assessing local impacts, the SETAC (Society of Environmental Toxicology and Chemistry) recommends the use of the Site dependent approach. This approach allows considering, in the characterization step, some spatial and temporal conditions of releases and some characteristics of the potentially affected environment in order to realize fate, exposure and effect analysis.

Our methodological research focuses on fate analysis step and its integration in the classification step. The novelty of our approach is the consideration of local environmental characteristics, through the USEtox model in a modified version, to locally assess toxicity and odours impacts. USEtox is a multimedia model which describes the fate, the exposure and the toxic effects of substances released in the environment through three boxes (global, continental and urban). For spatial assessment of substances fate, we propose to change in the dimensions of the environmental boxes, compartments and in local relevant parameters which are determined by default in USEtox and not suitable for local assessment.

This work leads to the construction of a new set of spatial elements for classification and characterization steps for toxicities and odours impacts. The new classification appears necessary for local issues such as toxicity and odours. To solve the problem of assessing local impacts, the SETAC (Society of Environmental Toxicology and Chemistry) recommends the use of the Site dependent approach. This approach allows considering, in the characterization step, some spatial and temporal conditions of releases and some characteristics of the potentially affected environment in order to realize fate, exposure and effect analysis.

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This work leads to the construction of a new set of spatial elements for classification and characterization steps for toxicity and odours impacts. The new classification step will be based on USEtox results from the new version and the consideration of spatiotemporal conditions of emission. The characterization step will be limited to the consideration of health effect and olfactory thresholds. The final objective is to compare classical LCA results (without taking into account spatial and temporal parameters) with located toxicity and odour LCA results. The spatial assessment of local impacts should allow policy makers and stakeholders to get “personalized”answers to debate issues.

EMO2C-3
Coupled modelling of plant uptake, soil water balance and soil solute transport for estimating the fate of cadmium and lead in amended agrosystems
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The novelty of our approach is the consideration of local environmental characteristics, through the USEtox model in a modified version, to locally assess toxicity and odours impacts. USEtox is a multimedia model which describes the fate, the exposure and the toxic effects of substances released in the environment through three boxes (global, continental and urban). For spatial assessment of substances fate, we propose to change in the dimensions of the environmental boxes, compartments and in local relevant parameters which are determined by default in USEtox and not suitable for local assessment.

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The concentration in the pore water was calculated assuming a linear sorption isotherm assuming that sorption is proportional to organic matter.

Concentration maps were calculated at a resolution of 1x1 km2 for the whole area of annual crops in each of the regulatory zones North, Centre and South. Simulations including the Cramer and the dry bulk density that a spatial 95th percentile has to be selected for obtaining an overall 90th percentile when median or geometric values of these substance properties are used as input for the scenario calculations in the regulatory process. So the target was to select a spatial 95th percentile. Scenario were selected based on maps of the peak concentrations in total soil and in pore water that were generated for 19 substances and averages depths of 1 and 20 cm. The selected scenarios for concentration in total soil have all relatively high organic matter contents and low temperatures. However, scenarios for the pore water concentration have all relatively low organic matter contents because the pore water concentration is almost directly proportional to the inverse of the organic matter content for substances that show significant sorption.

The aim of the exposure assessment was to obtain a 90th percentile of the PEC in space and time considering all fields in the regulatory zones North, Centre and South grown with the target crop where this active substance is applied. The types of ecotoxicologically relevant concentrations considered were both the concentration in total soil and the concentration in pore water averaged over the top 1, 2.5, 5 or 20 cm of soil (both peak and TWA concentrations for time windows up to 56 d). The scenario selection was based on a simple analytical model that calculates the concentration in soil assuming that degradation (first-order kinetics at a constant rate) is the only loss process from the 20-cm plough layer. The concentration in the pore water was calculated assuming a linear sorption isotherm assuming that sorption is proportional to organic matter.

Concentration maps were calculated at a resolution of 1x1 km2 for the whole area of annual crops in each of the regulatory zones North, Centre and South. Simulations including the Cramer and the dry bulk density that a spatial 95th percentile has to be selected for obtaining an overall 90th percentile when median or geometric values of these substance properties are used as input for the scenario calculations in the regulatory process. So the target was to select a spatial 95th percentile. Scenario were selected based on maps of the peak concentrations in total soil and in pore water that were generated for 19 substances and averages depths of 1 and 20 cm. The selected scenarios for concentration in total soil have all relatively high organic matter contents and low temperatures. However, scenarios for the pore water concentration have all relatively low organic matter contents because the pore water concentration is almost directly proportional to the inverse of the organic matter content for substances that show significant sorption.

Calculations with the simple analytical model for a number of example substances showed that the concentration in total soil increased in the order South-Centre-North and that the concentration in the pore water increased in the order North-Centre-South (so opposite orders for these two types of concentration).

EM02C-6
Mechanisms behind conservatism in tiered soil risk assessment
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In recent EFSA opinions on assessment of exposure of organisms to substances in soil a rigorous scientific concept was presented to describe the spatio-temporal distribution of chemical substances in soil. Further ecologically relevant soil layers were derived. These layers represent the habitats of specific soil organisms used for risk assessment. For example the soil layer 0 cm is proposed for epigeic and anecic earthworms. The mean concentration for this layer (Ecologically Relevant Concentration) can be identified from the predicted concentration depth distribution. It is finally used for lower tier risk assessment (RA) in combination with an effect endpoint, e.g. a NOEC. Though this approach appears straightforward it produces a surprising result. Compared to current soil RA it leads to dramatically increased ERC for a number of test organisms. On the other hand there are several publications comparing the current lower tier soil RA to higher tier (field) studies serving as reference tier. These came to the conclusion that the current soil RA is appropriate, i.e. it discriminates between critical and uncritical substances in sufficiently conservative way. Why does the soil RA proposed by EFSA become so conservative and is this justified? A concept is proposed which analyses the whole process (design of lower tier effect study, derivation of endpoint, link to the exposure situation under real-world conditions) in order to provide answers to the above question. The RA for earthworms is used as example because of the large number of studies available. Several factors may be considered to attribute to the different occurrence of effects in reference tier (field) study.
- Time to effect. Duration of study at reference tier typically 1 year, usually 56 days at lower tier (earthworm reproduction).
- Likelihood of proposed ecologically relevant soil layer, e.g. 0-1 cm. This habitat is very narrow. Extremes of soil moisture and temperature may render it temporarily to a hostile environment.
- Impact of initial concentration. NOEC values usually in terms of initial concentration. Often experimental period at reference tier is long compared to DT50 of compound. Thus mean concentration is likely to be much lower than initial one.

These factors are investigated employing detailed analysis of the spatio-temporal concentration distribution in soil. Based on this information local effects can be estimated and aggregated on population level.

EM02D-1
Variability and estimation of pesticide half-lives in vegetation
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9IVYM, Bildhoven, Nederland
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12Institute of Soil Science and Plant Nutrition, Wageningen, Nederland
13Centre-South (so opposite orders for these two types of concentration).
The one-dimensional non-steady state MACRO model was used to help interpret observed propyzamide and carbetamide losses in an 8.6 ha underdrained headwater sub-catchment of the Upper Cherwell, with strongly undulating topography. Although the catchment is dominated by heavy clay soil, a small area of lighter and better drained soil over sandstone generates some groundwater contribution. Estimates of recharge to the sandstone aquifer were made using soil moisture balance calculations.

A one-dimensional time-variant groundwater model was used to represent herbicide transport. Significant transfers of both herbicides to the drain network occurred soon after application. Peak concentration coincided with increased drain flow, but both herbicides were extensively removed before entering the Meuse river. Hydrograph and chemograph recession were also reasonably well predicted. Nearly 100% of herbicide transport to drainings in this heavy clay soil is predicted to occur via macropore flow for both propyzamide and carbetamide. The results demonstrate the utility of one-dimensional models as an explanatory framework for processes operating in larger undulating fields with significant topography. The results also confirm that drainflow is probably the dominant pathway for the transfer of these herbicides to the catchment outlet. This imposes considerable constraints on the management options available to reduce problematic herbicide exposure in this catchment.

EM02D-3
Nano-pesticides: state of knowledge, impacts on fate and exposure assessment

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Nano-formulations are already used in the pharmaceutical and food industries. In comparison, applications in the agrochemical sector are only emerging and a rapid growth is predicted in the upcoming years. Initial application of nanoparticles as within agricultural practises could be one of the rare intentional diffuse inputs of engineered nanoparticles into the environment (e.g. nano-silver). It is thus essential that risks and benefits to human and environmental health are adequately evaluated.

The aim of the present study is to (i) review the current state of knowledge on nano-pesticides, (ii) identify possible impacts on environmental fate and (iii) analyse the suitability of current pesticide exposure assessment procedures to account for novel properties within the EU regulatory context.

Nano-pesticides encompass a great variety of products and cannot be considered as a single category. The aims of nano-formulations are generally common to other pesticide formulations and consist in increasing the apparent solubility of poorly soluble active ingredient (a.i.), releasing the a.i. in a slow/targeted manner and/or protecting against premature degradation. Nano-formulations are expected to have significant impacts on the fate of a.i. and/or to introduce new ingredients whose environmental fate is still poorly understood.

Current exposure modelling procedures are probably satisfactory for assessing the fate of most nano-formulations, provided that (i) realistic sorption and degradation parameters can be determined and used as model inputs, (ii) changes with time can be accounted for, if necessary, or (iii) the separate assessment of the various ingredients is proved by the same approach. The potential retarded/facilitated transport of the a.i. due to its association with a nano-carrier (e.g., polymer based or nano oxides associated with a.i.) should also be considered.

Further research is thus required in order to (i) identify the assumptions currently applied that are not valid in the case of nano-pesticides, (ii) evaluate the points or situations in which differences may impact significantly on the exposure assessment outcomes, and (iii) refine or adapt current protocols as required.

EM02D-4
On the effect of local pesticide reduction programmes in large river basins

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Large river basins have multiple sources of pesticides, which may be spread over more than 100-1000 km2. To effectively reduce the amount of pesticides entering surface water bodies, local reduction programmes in upstream areas can compensate downstream water use e.g. raw water quality for drinking water abstractions. Local reduction programmes that target certain uses of pesticides may clearly affect local concentrations while the expected impact on the larger scale may fail to appear. For larger scale assessments pesticide fluxes coming from other sub basins and even transnational fluxes need to be taken into account. We illustrate the effect of a local reduction programme for the use of glyphosate on hard surfaces in the Meuse river basin. We used a long-term data set of glyphosate concentrations in the Meuse river to derive the trends in the concentrations before and after the implementation of the reduction programme. The results indicate spatial difference in the course of concentrations over time. In some areas of the river basin trend reversal is found, which might be possibly linked to the association with a nano-carrier (e.g., polymer based or nano oxides associated with a.i.) should also be considered.
Furthermore setting leaching studies into context of the area of use are explored by GIS analysis. The approach may support the evaluation of leaching studies generated under certain conditions in one region by setting them into context (on a relative scale) to the environmental conditions in other regions.

4. References

EM02D-6
PEARL BE: combining a meta-model and a process-based pesticide leaching model to develop groundwater scenarios for Belgium
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Registation of pesticides on the European market requires a risk assessment of groundwater contamination following guidelines of the FOCUS workgroup. Nine worst-case standard FOCUS scenarios representing different soil types and climatic conditions are developed for Europe. In this study, the pertinence of standard FOCUS scenarios for Belgium was assessed and more specified for each agricultural region using local information on soil and climate. Local soil profiles were constructed using information from the Aardewerk-database (13,000 soil profile descriptions for Belgium) and the digital Belgian soil map. Within each agricultural region, soil profiles were parameterised using median soil properties for each soil type. Hydraulic properties were determined using pedotransferfunctions derived for Belgian soils. Daily meteo data for a 20-year period were available for 10 by 10 km grid cells. Crop calendars were parameterised according to the FOCUS Climatic scenario, Modelling of pesticide leaching was done in a two-step approach. First Meta-PEARL was applied to all soil profiles to delineate vulnerable soils using median yearly net precipitation for each agricultural region. Soils with a predicted groundwater concentration close to the 80th percentile of PECs were considered as vulnerable soils. Next FOCUS PEARL was applied to the selected sensitive soils using daily meteo data for the centroid of each agricultural region. At the scale of Belgium, the predicted pesticide groundwater concentrations for 10 sensitive soils were compared to the results of 4 FOCUS scenarios (Châteaudun, Hamburg, Kremsmunster, Okehampton) to evaluate the pertinence of the FOCUS scenarios for Belgian conditions. Spring and autumn application of FOCUS substance B to sugar beet and winter cereal were compared. The Belgian scenarios were more critical than the FOCUS scenarios in all cases. At the scale of the agricultural region, FOCUS PEARL was applied to 3 sensitive soils and the scenario with the highest PEC was selected as the sensitive local scenario for the region. The study shows that the results from the FOCUS scenarios are quite different from the results obtained with scenarios adapted to the local situation and based on more detailed information on soil characteristics. For Belgium, the use of local scenarios derived according to FOCUS guidelines is more conservative with respect to the protection of groundwater resources.

EM03 - Global scale modeling of environmental cause-effect chains for risk assessment and life cycle assessment: Quo vada?
EM03-1
GIS-based regionalized Life Cycle Assessment: how big is small enough? Methodology and case study of electricity generation
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A few regionalized life cycle impact assessment method developers have justified their choice of spatial scale. In order for the promise of regionalized impact assessment to be realized, spatial scale should be chosen in a systematic fashion, and should match the spatial scale of the environmental phenomena that drive changes in characterization factors across space.

The purpose of minimizing the global spatial autocorrelation (AC) as a systematic technique to choose the spatial scale of impact assessment methods. Spatial autocorrelation is present when a data value can be inferred with minimal error from the data values of its spatial neighbors. The presence of AC can be interpreted as the phenomenon which are influencing AC on a different spatial scale than the chosen one. The minimization of autocorrelation is therefore the choice of a spatial scale which best matches the spatial scale of the environmental or societal phenomena which are driving significant changes in CFs. We calculated changes in global spatial autocorrelation at various spatial scales by using different techniques to aggregate disaggregated data. Our hypothesis is that the minimal spatial autocorrelation can be found by varying the number of spatial areas in the impact assessment method. In a case study for ecosystem damage due to freshwater consumption, global spatial autocorrelation was minimized at a spatial scale of 12,000 spatial units. The choice of spatial scale is quite important in understanding, interpreting, and applying regionalized impact assessment methods correctly and with minimal uncertainty. Because the method proposed here makes no assumptions about spatial pattern or number of spatial areas, it can be applied to all regionalized impact assessment methods.

EM03-2
Spatially-explicit global scale modelling of acidifying air emissions
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Spatially-dependent freshwater concentration-species richness response functions for nitrogen and phosphorus
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Up to now, there are no concentration-species richness response functions for nitrogen (N) and phosphorus (P) in freshwater systems on a global scale. Reduced data availability and strong spatial specificity are reasons that challenge global scale modelling. The objective of our study is to (1) derive dose-response functions of aquatic species richness along both N and P concentration gradients for freshwater ecoregions worldwide and (2) compare the potential gain in precision and the increase in uncertainty by simulating different levels of spatial detail.

This was attained by gathering data from the literature (total of 472 studies) on the occurrence of aquatic species (diatoms, invertebrates, and bacteria, among others) along N and P concentration gradients. We gathered data on a total of 3707 species distributed across 110 freshwater ecoregions. In the presentation, we will (1) show results of the logistic regressions based on the empirical data we attained from the literature and (2) address the issues of increasing uncertainty and loss in precision by grouping ecoregions with similar characteristics with each other. This is a necessary step to assess the risk of nutrient pollution to world's regions for which very little data is available. Our work will contribute reduced data availability by performing a thorough empirical data gathering on a global scale. It also maintains spatial specificity and with the use of an ecoregion level of spatial resolution. Ultimately, our study provides a consistent method to compare the effects of N and P concentration to different aquatic organisms and different freshwater systems worldwide. Our results allow for ecologists to identify world's freshwaters that are most susceptible to nutrient concentration shifts. In addition, it is a valuable method to environmental risk and life cycle impact assessments since our results can be easily integrated to fate and exposure modelling so as to provide a cause-effect chain of freshwater eutrophication.
Including metal speciation in LCA terrestrial ecotoxicity: new regionalization character factors

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At present, ecotoxicological impacts of metals are often the main contributors to LCA results. This domination does not necessarily reflect a real toxicity but rather indicates the misuse of fate and effect models, initially developed for organic compounds and not accounting for metal speciation. A framework for including metal speciation in freshwater ecotoxicity in LCA has been recently developed. It relies on the use of a commercially available speciation model to define the bioavailable metal fraction and of freshwater archeotypes to account for the influence of freshwater physicochemical properties on speciation. Although really interesting, this approach is not directly applicable to soils because speciation models were developed for aquatic environments. Soils greater heterogeneity hinders generalization and could invalidate hypothesis made in aquatic geochemical modelling.

The main goal of this project is thus to develop a method specific to soils, including defining the appropriate regionalization scale, in order to obtain terrestrial ecotoxicity CFs.

The Harmonized World Soil Database (HWSD) version 1.1 (FAO/ILASA/ISRRC/SSS-CAS/JRC, 2009) was used and enhanced to gather data on soil properties. The soil bioavailable metal fraction is computed with a soil specific parameterization of two available aquatic speciation models (WHAM 6.0 and MINTEQA+), for each of the 5200 possible combinations of soil properties of the world listed in the enhanced HWSD. The model parameterization is validated using field data. Multiple linear regressions are performed to detect the most influential soil properties on bioavailable metal fraction and soils are grouped in archeotypes according to these properties. According to the framework proposed for freshwater ecotoxicity, a bioavailable factor is added to the definition of the CF and new effect factors are computed in terms of bioavailable fraction. Using the new regionalization scale here defined, new CFs are calculated for Zn, Ni and Cu for terrestrial ecotoxicity.

Results obtained are used to indicate that it is possible to group soil types of the world in 70 different archeotypes when considering the 9 most influential soil properties on speciation (R2=0.776). Model predictions are in good agreement with measured values (a difference of no more than one order of magnitude). Zinc bioavailability factors, with a variability of 20 orders of magnitude, show the importance of integrating speciation in LCA.

Evaluation of spatial variability of metal bioavailability in soils using geostatistics

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Soil properties show significant spatial variability at local, regional and continental scales. This is a challenge for life cycle impact assessment (LCIA) of metals, because fate, bioavailability and effect factors are controlled by environmental chemistry and can vary orders of magnitude for different soils. Here, variography is employed to analyse spatial variability of bioavailability factors (BFs) of metals at the global scale. First, published empirical regressions are employed to calculate BFs of metals for 7180 soil profiles. Next, geostatistical interpretation of calculated BFs is performed using ArcGIS Geostatistical Analyst. Results show that BFs of copper span a range of 6 orders of magnitude, and have significant spatial variability at local and continental scales. The model nugget variance is significantly higher than zero, suggesting the presence of spatial variability at lags smaller than those in the data set. Geostatistical analyses indicate however, that BFs exhibit no significant spatial correlation at a range beyond 3200 km. Because BF is spatially correlated, its values at unsampled locations can be predicted, as demonstrated using ordinary krigging method. Similar approach can be employed for analyzing spatio-temporal variability of terrestrial ecotoxicity characterization factors of metals. Predicted maps can be used to provide a set of regionalized factors at spatial scales that are both scientifically relevant and practically feasible in LCIA.

A global assessment of the temporal and spatial variability of national dilution factors

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Of the many factors which influence the exposure of the freshwater aquatic environment to contaminating chemicals none has a more dramatic effect than dilution. Both where and when a chemical enters surface water can make an enormous difference to its impact on wildlife. This is of particular importance for down-the-drain"chemicals as these substances are discharged to freshwaters via sewer systems after consumer use. However, too often, this dilution capacity is fixed to a "generic" value. Although the spatial variability of dilution factors is often acknowledged, temporal variability is often unaccounted for which may potentially lead to underestimating the environmental risk. Geostatistical interpretation of these dilution differences across the world, estimates of dilution factors were developed globally at a 0.5 degree resolution using gridded data. Thus, the focus here is on the numbers and location of the human population and the river water available to dilute their waste. The river flows estimates are calculated at both annual and monthly resolution based on readily available annual and monthly runoff estimates. The domestic waste water effluent is derived from combining gridded population and national per capita domestic water use estimates. For each grid cell both annual and monthly dilution factors were generated. This approach allowed the quantification of temporal and spatial variability of dilution factors not only at a catchment level but also at a national level, by means of statistical measures such as median and percentiles. This method revealed the dramatic differences in available dilution of chemicals both within and between countries, for example Canada has on average 4- orders of magnitude more dilution available than Tunisia, and Finland 3-orders of magnitude more than Spain. Over the course of a year, national dilution could vary between 10 and a 1000-fold depending on the country.

The work presented here is a significant step forward in terms of understanding the impact of river flow temporal variability on dilution factors at a national and global scale. The proposed methodology has great potential for scientists and decision makers across the globe, as it provides the means to improve screening-level chemical risk assessments.

Lead and lead isotopes in agricultural soils of Europe: natural distribution or contamination?

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In environmental sciences the 206Pb/207Pb isotope ratio is routinely used to argue for Pb contamination of different compartments of the environment. However, the lead isotope background variation of a continent has never been established for any sample material. For the first time, a map of a Pb isotope landscape, based on samples of agricultural soils at the continental-scale, is presented. Agricultural soil samples (Ap-horizon, 0-20 cm), collected at an average density of 1 site/2500 km2 (2211 samples in total) from 33 European countries, were analysed for Pb concentration and Pb isotopes (206Pb, 207Pb, 208Pb). Lead concentrations vary from 1.6 to 1309 mg/kg, with a median of 16 mg/kg. Isotopic ratios of 206Pb/207Pb range from 1.116 to 1.727, with a median of 1.202. The new data define the soil geochemical Pb background for European arable land, providing crucial information for geological, environmental and forensic sciences, public health, environmental policy and mineral exploration. Pb concentrations and Pb isotopes show a high variability dominated by lead and influence by climate. Lead concentration anomalies mark most of the known mineralised areas in Europe. At the continental scale of this survey, it is difficult to distinguish between natural anomalies due to ore occurrences, and an amplified signal caused by mining and smelting. With the exception of some very local anomalies (related to cities, smelters), none of the observed patterns can be directly related to contamination, and all anomalies rapidly decrease to background values with distance to source. Combining the regional distribution of Pb concentrations with Pb isotopes, the conclusion is that the majority of lead in European agricultural land is still of natural origin.

EP01 - Antimicrobial resistance in the environment

EP01A-1 Characterization and comparison of multi-drug resistant methicillin-resistant Staphylococcus aureus [MRSA] from recreational beaches and high touch surfaces at a university and surrounding community

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Over the last decade community-acquired methicillin-resistant Staphylococcus aureus [MRSA] has emerged as a major cause of disease in the general population with no source (related to cities, smelters), none of the observed patterns can be directly related to contamination, and all anomalies rapidly decrease to background values with distance to source. Combining the regional distribution of Pb concentrations with Pb isotopes, the conclusion is that the majority of lead in European agricultural land is still of natural origin.

EP01A1-1 Characterization and comparison of multi-drug resistant methicillin-resistant Staphylococcus aureus [MRSA] from recreational beaches and high touch surfaces at a university and surrounding community
local community (n=130). The presence of type I of mobile Staphylococcal Cassette Chromosome SCCmec, multilocus sequence typing [MLST] of the allelic profile of seven housekeeping genes, and the presence of aminoglycoside resistance gene, aadD; macrolide resistance genes, erm(A), erm(B) and ermA(C), and msr(A); and tetracycline resistance genes, tet(M), tet(K), were determined by PCR assays and sequencing. Pulse field gel electrophoresis was done and the genetic relatedness of the isolates to USA100 was done by Dice coefficient, UPGMA using the GelCompar II software. Strains that had > 80% homology with USA100 were classified as USA100. The highest level of MRSA positive samples ([5.5%] were found in fresh water running into the marine beaches, where we frequently observed children playing during sampling, and at the fresh water beach on Lake Washington, while 11.7% of the surfaces from 5 of 8 undergraduate homes were MRSA positive. A surprising 98% of the 55 MRSA isolates were resistant to other classes of antibiotics and most likely represent reservoirs for these genes in the environment. In North America the majority of community acquired MRSA infections are due to USA100. This clone was found at the UW, in student housing and in the community but not in the recreational beach samples.

EP01A-2

Unique distribution of sulfonamide resistance genes, sul, in the Philippines aquatic environment

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EP01A-3

Tetracycline and mercury resistance genes in aquaculture sediments


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Farming fish in cages is an open system which has an effect on the chemical and ecological balance of the farming area. Fish feed, medicines and anti-foulants can affect microbial communities in the sediments under the cages. Considerable part of the substances used ends up to the water outside the actual aquaculture facility either directly or via excremen of the fish. The aim of this study was to characterise the effects of aquaculture to the surrounding waters and sediments. In particular we were interested amount and diversity of tetracycline resistance genes (tet) and mercury resistance (merA) in comparison to pristine sediment. Four fish farms in the Finnish and Swedish archipelago were sampled during years 2006-2011. Total DNA from top sediment samples was isolated with Fast DNA spin kit. Quantification of bioavailable tetracyclines and mercury was done by bioelevator bacteria. Total tetracycline content by was analysed by HPLC and total mercury by ICP-MS The resistance genes (tetA, tetC, tetH, tetM and merA) were quantified by qPCR. No bioavailable tetracycline or mercury was found in any of the samples before 2011. LODs were 0.3 µg g⁻¹ sediment for tetracycline and 0.04 ng g⁻¹ sediment for mercury. The total amount of mercury, tetracycline and oxytetracycline was very low in all samples taken before 2011. However, there was high amount (more than 2 ng g⁻¹) of oxytetracycline present in one sample taken on 2011. The number of tetracycline resistance genes was clearly elevated in all fish farm samples until 2009 and the number of mercury resistance genes was also slightly elevated. Samples taken after that are currently under analysis. Diversity of tetC gene was non existent and diversity of tetM was relatively low. In contrast, the diversity of merA was considerable. The aquaculture farms had used tetracyclines but ceased that about a decade ago so the reasons for the elevated tetracycline resistance genes is not obvious, like are the accumulation of tetracycline resistance genes may implicate a common source. For the genes. The merA sequence diversity in both fish farm samples was similar, phylogenetic analyses showed that there was a little difference between sequences from different sampling sites.

EP01A-4

Abundance of streptomycin and tetracycline resistance genes in apple orchards treated with streptomycin in comparison to untreated apple orchards

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Streptomycin is the only antibiotic authorized for use in plant agriculture within the EU and Switzerland. Its use is authorized on an annual basis for the prophylactic treatment of apple and pear orchards against the bacterial disease fire blight. Tetracycline is currently the only other viable alternative resistance should resistance to streptomycin emerge in the fire blight pathogen; Erwinia amylovora. We have developed a multiplex qRT-PCR for the relative quantification of streptomycin and tetracycline resistance genes, with the 16s rRNA genes as the endogenous control. Using these multiplex qRT-PCRs we have monitored the abundance of streptomycin and tetracycline resistance genes in apple orchards treated and untreated orchards in 2010 and 2011.

2. Materials and methods

Samples of flowers, leaves and soil were collected from three orchard sites at each time-point (265 samples per year). The time-points consisted of prior to streptomycin spraying, one day after the streptomycin spraying, two weeks after streptomycin spraying and at apple harvest. The DNA extraction method and the relative abundance of resistance genes were detected as previously described.

3. Results and discussion

The abundances of strA and strB genes increased in the flower and leaf samples over time in comparison to the untreated samples in 2010 and 2011. However, the harvest samples contained a similar abundance of strA and strB to the samples prior to streptomycin spraying. There were no streptomycin influenced changes in the abundance of streptomycin resistance genes in the soil samples. The relative abundances of the tetracycline resistance genes tetB, tetM and merA were not affected by the treatment with streptomycin in the flower, leaf or soil samples. There were low fluctuations within the abundances of the streptomycin and tetracycline resistance genes within the samples isolated from the untreated orchards over time.

4. Conclusions

There were zero increases in the abundances of strA and strB genes associated with streptomycin treatment in the flower and leaf samples. However, the abundance of these resistance genes returned to pre-treatment level at harvest. Streptomycin treatment did not influence the abundance of streptomycin resistance genes in the soil samples nor did it influence the abundance of tetracycline resistance genes within the orchard samples. Thus, the streptomycin associated increases in resistance gene abundance are temporary.

EP01A-5

Antimicrobial resistance and their selective in pig manure and sewage sludge

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Antimicrobial resistance is a pestering problem, and the solution still remains to be found. Due to a supposed reservoir function of the environment, environmental contamination with antibiotics and/or resistant bacteria is a serious issue. In addition, bacteria are hard to trace down, once spread into water or soil. Therefore, it is the more necessary and promising efforts before they enter the environment.

We investigated the antimicrobial resistance of bacteria - comprising E. coli, enterococcus, lactobacilli and clostridia - which were isolated from sewage sludge and pig manure. The statistical association of antimicrobial resistance with different potentially selective factors was assessed in a linear model. Chemical precipitation in sewage plants was significantly associated with increased bacterial resistance in sewage sludge. Antibiotic contamination and heavy metal contents in pig manure were significantly associated with bacterial resistance in pig manure. E. coli from manure samples with supermedian contents of copper (> 11.8 mg/kg manure wetweight) were significantly more often resistant against ampicillin and piperacillin. E. coli from manure samples with zinc contents > 22.75 mg/kg manure wetweight were significantly more often resistant against ampicillin, doxycycline and piperacillin.

In order to track whether environmental isolates might be transferred to humans, we investigated by ERIC-PCR the genetic relatedness of E. coli from pig manure, sewage plants, and human hospital samples. In general, different antibiotic groups prevailed among isolates from pig manure and human hospital isolates; isolates from sewage sludge resembled partly isolates from pig manure, partly isolates of human hospitals. Two closely (or clonally) related E. coli isolates were found in a sample of pig manure and a human stationary patient. Interestingly, despite the close phylogenetic relatedness, both isolates differed significantly in the resistance genes. On the contrary, identical resistance gene profiles were present in phylogenetically unrelated isolates from humans and pigs. These findings indicate a major role of horizontal spread in the distribution of antimicrobial resistance among E. coli.

EP01A-6

Occurrence and dissemination of antibiotic resistance genes in anthropic environments


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Dispersal of strain resistance genes is recognized to occur in various environmental compartments although it remains difficult to demonstrate in complex environmental matrices. Some environments have been defined as putative hot spots for gene transfer as they can sustain high microbial cell densities and combine both antibiotic and antibiotic resistance bacteria. In this study we compare the occurrence of class 1, 2 and 3 integrons (mobile genetic elements mobilizing different resistance cassettes according to their respective class) from wastewater, sludges, farm slurry and manure. Same or equivalent environmental matrices were also evaluated for their propensity to support the transfer of a model integron-bearing plasmid (pB10). In farm samples, only class 1 and class 2 integrons could be detected. Prevalence of integrons was significantly more important in the farmyard manure than in the slurry. In
WWTPs influential, the occurrence of class 1 integrons is significantly more important than the other classes. We could show that WWTPs as a process reduced the relative concentrations of integrons by 2 log. In dynamic point of view, plasmid pB10 did not appear to disseminate in farm manure microcosms while maintaining steadily over concentrations used as action limits in environmental risk assessment (ERA) may exert a selective pressure on bacteria of importance to public health in the environment.

Regarding integrons, the difference of occurrence may reflect more or less homogenous anthropic selective pressure on antibiotic resistances. On the other hand, propensity for pB10 to transfer sludge microorganisms seems to rely on the origin of the sludge ecosystem from a given treatment process.

EP01B-1
Detecting evolutionary hot spots of antibiotic resistances in Europe (DARE)
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Antibiotic resistances (ARs) are a great threat to human health, which has meant that research in this area has focused primarily on their role within clinical settings. This is in strong contrast to the research in nonclinical environments, such as the natural and urban environment, where this research has only recently received more attention. Here the first results of a European wide network COST Action are presented. The Action has involved 84 members of 18 European Nations and Israel which try to identify the gaps of knowledge, which need to be investigated in order to propose interventions to curb the spread of AR evolution and AR microbes within the environment. The Action has developed criteria for antibiotics and bacterial organisms and environmental hot spots of AR evolution, which should be investigated as first priority. Furthermore, in order to assess the risk of antibiotic resistance evolution a new approach of risk assessment is needed. However, the Action has also identified here the largest gap of knowledge.

Currently, no systems exist which will allow to assess the risk of spread or evolution of new antibiotic resistances. Therefore investigations to fill this gap of knowledge are strongly called for and this is also the case for research resulting in a first geographic pattern on AR resistance in Europe. Such an epidemiological base information exists already for clinical and veterinary data, but is missing for environmental data.

EP01B-2
Selective pressure of antibiotic pollution on bacteria of importance to public health
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Many bacteria of clinical importance survive and under certain conditions may grow in different environments. Antibiotic pollution may exert on them a selective pressure leading to an increase in the prevalence of resistance. The objective of this study is to determine whether measured environmental concentrations (MEC) of antibiotics and concentrations used as action limits in environmental risk assessment (ERA) may exert a selection pressure on bacteria (ERA) may exert a selection pressure on bacteria.

Species sensitivity distributions were derived for ciprofloxacin, erythromycin and tetracycline using endpoints calculated from minimum inhibitory concentration distributions of clinically relevant bacteria. The potentially affected fraction (PAF) of bacterial taxa at MEC of antibiotics and ERA action limits was used as a proxy for antibiotic selective pressure. MEC and ERA action limits were also directly compared to wild-type cut-off values. The PAF of bacterial genera at antibiotic concentrations measured in water environments is estimated not to exceed 6%. MEC in river sediments, swine feces lagoons, liquid manure and farmed soil are estimated to inhibit wild-type populations in up to 49%, 84%, 100% and 23% of bacterial genera, respectively. At concentrations used as action limits in ERA, erythromycin and ciprofloxacin are estimated to inhibit wild-type populations in up to 15% and 65% of bacterial genera. Concentrations of antibiotics measured in different environments and used as action limits in ERA are high enough to exert a selective pressure on clinically relevant bacteria that may lead to an increase in the prevalence of resistance.

EP01B-3
Monitoring and management of antibiotic resistance of veterinary drugs in Germany
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The use of antibiotic drugs in veterinary therapy results in a target conflict of demand for therapy vs. antimicrobial resistance. Advantages such as secure animal health of companion and farm animals, avoidance of economical losses in animal husbandry and prevention of bacterial zoonoses are opposed by the risk of the enhancing antimicrobial resistance of bacterial pathogens. A management approach is the responsible use of antimicrobials applying the "ONE HEALTH Principle" (animals + humans = one health) of the WHO and EU (animal health strategy 2007-2013). This strategy needs information on the current status of antimicrobial resistance. This information is obtained from monitoring programs conducted and improved in Germany for more than 10 years.

These include:
National resistance monitoring - animal pathogens
National food monitoring program
Monitoring of resistance for zoonosis

National Monitoring of resistance for commensal

Additional information is obtained from the collection of postmarketing data (ADRs, PSURs etc.) and since 2011 from data of antibiotic disposal to be registered by law. This talk presents some of these data and shows management actions drawn from these data and put into force both nationally and within the whole EU.

EP01B-4
Reduction of pathogens and antibiotic resistance genes by Soil Aquifer Treatment (SAT) in the Mézquital Valley, Mexico
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Aquifer recharge projects provide advantages for water management, namely, treatment of reclaimed water and additional dilution of pollutants due to mixing with natural groundwater. Nevertheless, this practice can represent a health and environmental hazard because of the presence of pharmaceuticals, pathogens and antibiotic resistance genes. The Mézquital Valley, 60 km north of Mexico City, is the world's largest wastewater (WW) irrigation area. The WW from Mexico City is transported without treatment to the Mézquital. There, approximately 800-900 km² of agricultural land are irrigated with this untreated WW. We followed the WW on its way to the Mézquital taking samples from seven sites starting with the effluent of a big hospital in Mexico City and ending with water from a spring that arises after SAT in the Mézquital. Total DNA was extracted from the water and applied to quantitate real-time PCR (qPCR) targeted to the 16S rRNA gene of bacteria, the 23S rRNA gene of Enterococcus and to six resistance genes mediating resistance to fluoroquinolones, sulfonamides and β-lactam antibiotics. Total bacterial load was reduced by almost three logs from 1.75 [GREQX] 109 to 16 S rRNA gene copies / 100 ml WW to a final concentration of 3.73 [GREQX] 106 S rRNA gene copies / 100 ml spring water destined for drinking water use. A four log reduction to 1.66 [GREQX] 103 copies / 100 ml WW was achieved for the nosocomial pathogen, Enterococcus. For the resistance genes, the highest concentration was found for the sulphonamide resistance gene sul1 with a concentration of 1.14 [GREQX] 109 gene copies / 100 ml WW, which decreased to 1.38 to 104 copies in the spring water. The fluoroquinolone resistance genes qnrA and qnrB were present in the WW at 5 x 105 copies / 100 ml, both were completely eliminated on their way to the spring. In summary, two of the six resistance genes were completely eliminated, the others were reduced by three to five logs from the WW to the spring. Mixing of WW with rainwater, passage through a dam, and sedimentation of particles suspended in WW hardly affected bacterial load and resistance gene abundance. The major cleaning effect was exerted by SAT in the Mézquital. Natural WW treatment, like sedimentation, mixing with rainwater and SAT, can considerably decrease biological contaminants in water. However, total bacteria, nosocomial pathogens and resistance genes were not satisfactorily removed to use this water without further treatment as drinking water.

EP01B-5
Impact of treated wastewater irrigation on antibiotic resistances in agricultural soils
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Antibiotic resistance (AR) is a global phenomenon with severe epidemiological ramifications. It is becoming increasingly clear that anthropogenically-impacted natural aquatic and terrestrial “hotspots” may be playing an important role in the global persistence of AR. These “hotspots” may play a critical role as gene reservoirs which can contribute to clinically relevant (potentially pathogenic) organisms via water and food webs. Treated-wastewater (WW) irrigation is becoming increasingly prevalent in arid regions of the world, due to growing demand and decline in freshwater supplies. The release of residual antibiotic compounds, antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs) from wastewater effluent may result in proliferation of AR in irrigated soil microcosms. The aim of this study was to assess the impact of WW-irrigation on soil AR and ARG reservoirs. Tetracycline, erythromycin, sulfonamide and ciprofloxacin resistance was assessed in agricultural soils irrigated in tandem with either freshwater (FW) or WW, using standard culture-based isolation methods and culture-independent molecular analysis using quantitative real-time PCR (qPCR). Significant.
levels of native bacterial antibiotic resistance were detected in both FW- and TWW-irrigated soils. Nonetheless, ARB and ARG levels in TWW-irrigated soils were on the whole identical (or sometimes even lower) than the FW-irrigated soils, despite detection of relatively high levels of both ARB and ARG in the TWW. This indicates that the high numbers of resistant bacteria that enter the soils from the TWW are not able to compete or survive in the soil environment; and suggests that the impact of the TWW-associated microorganisms in the soil microbiome is on the whole negligible. We therefore conclude that AR in the soil is primarily associated with native resistance of the indigenous soil microbiome and not with introduced strains or genetic elements.

**EP01B - Urban wastewater disinfection and solar irradiation of wastewater polluted stream: effect on antibiotic photo-degradation, inactivation of antibiotic resistant E. coli strains and their resistance**

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Two wastewater disinfection processes (UV radiation and chlorination) were tested in the inactivation of two previously selected E. coli strains and their resistance to three antibiotics (Amoxicillin (AMX), ciprofloxacin (CPX), and sulfamethoxazole (SMZ)). Next, disposal of urban wastewater treatment plant (UWWTP) effluent into surface water was simulated and the effect of solar irradiation on the photodegradation of target antibiotics, the inactivation of antibiotic resistant E. coli strains and their resistance were investigated. Two different strains of E. coli resistant to the target antibiotics (higher antibiotic resistance, HAR; lower antibiotic resistance, LAR) were isolated from wastewater sample and subsequently inoculated in previously autoclaved wastewater samples. Total inactivation by UV radiation (250 W wide spectrum UV lamp) was observed after 60 min in LAR E. coli strain inoculated wastewater (4.0 106 UFC/100 mL) but not after chlorination process. Additionally, unlike of chlorination process, UV radiation effect on resistance of LAR E. coli strain to CPX (MIC decreased from 12 to 8 µg/L). When simulated solar irradiation effect was investigated on wastewater polluted surface water, E. coli strains inactivation was found to be really low (26 and 50% respectively). According to wastewater disinfection tests, solar irradiation effect on resistance of LAR strain to CPX too, but MIC was decreased by 8 µg/L only after 180 min irradiation. Finally, the effect of solar irradiation on antibiotics (1 mg/L initial concentration respectively) photodegradation rate resulted in the following order (half-life time): CPX (t1/2= 24 min) < AMX (t1/2= 99 min) < SMX (t1/2= 577 min).

In conclusion, antibiotic resistance can be released into the stream from UWWTP effluents partially unaffected, and because of the lower efficiency of solar irradiation, the risk of horizontal transfer and selection of antimicrobial resistance genes among bacteria can increase.

**EP02 - Endocrine disrupting chemicals: recent developments**

**EP02A - Zebrafish eleutherembryos provide a suitable vertebrate model for screening thyroid gland disrupting chemicals**

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Thyroidal responses to androgenic diestruces were quantitated by the Thyroid Immunoassay Disruption Test (TIQDT) which was designed to provide a simple, rapid, alternative bioassay for assessing the potential of chemical pollutants and drugs to disrupt thyroid gland function. This study demonstrated that zebrafish eleutherembryos provided a suitable vertebrate model, not only for screening the potential thyroid disrupting effect of molecules, but also for estimating the potential hazards associated with exposure to chemicals directly impairing thyroxin (T4) synthesis. Aminolevulinic acid (ALA), potassium percarbonate, potassium thiocyanate, methimazole (MMI), phenol, polypropylene glycol and PCP were investigated. In the TIQDT performed on zebrafish eleutheroembryos, an estrogenic effect was recorded at levels of 0.05 and 0.1 mg/L for MMI and polypropylene glycol respectively. Concordance between TIQDT on zebrafish and mammalian published data was very high for those chemicals with a direct effect on the thyroid gland function, as the sodium-iodide symporter (NIS) and the thyroid peroxidase (TPO) inhibitors. The physiological relevance of T4-intracellular content was clearly higher than regulation at the transcriptional level of t,g or scla5a. Moreover, concentration-response analyses provided information about the thyroid disrupting potency and hazard of selected positive compounds. Finally, the effect of perchlorate, a NIS inhibitor, but not MMI, a TPO inhibitor, was completely rescued by low-micromolar amounts of iodide. TIQDT performed on zebrafish eleutherembryos is an alternative whole-organism screening assay that provides relevant information for environmental and human risk assessments.

**EP02B - Endocrine disrupting compounds as potential obesogens: musk compounds as a case study**


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The processes of sexual differentiation and development are dependent upon an intricate cascade of molecular signals and deviations from normal can have catastrophic effects on individual fertility and fitness. Previously, several studies have shown that exposure of Xenopus laevis to sufficiently potent estrogens at critical times during development results in feminization and/or demasculinization, including male to female phenotypic sex reversal at adequate doses. However, given that genotyping of X. laevis has only recently become possible, studies performed in the past were rarely able to make concrete linkages between genetic and phenotypic sex. Therefore, to further characterize the relationship between genotype and phenotype, X. laevis tadpoles were exposed to 0.1, 0.4 or 1.6 µg/L 17β-ethinylestradiol (EE2), the estrogen analog commonly used in oral contraceptives, from 12 h post-oviposition through 13 wks post-hatch. All EE2 treatments resulted in significant delays in time to metamorphosis. Genotyping showed that genetic sex ratios were similar among treatments. However, morphological evaluation revealed that phenotypic sex ratios were altered in all EE2 treatments. Interestingly, complete male to female phenotypic sex reversal was rare at the concentrations tested, and a surprising number of individuals displayed intersex gonads, abnormal gonads, and atypical vitellogenin over-expression, that were only diagnosed upon histological examination. The impacts of these conditions on fertility and fitness are not known but are likely to be adverse and more complex than complete sex reversal. In all likelihood, the relatively great number of intersex and abnormal animals is a result of estrogens functioning downstream of the initial molecular signals of sexual differentiation. Thus, genetically male animals receive mixed endogenous male and exogenous female signals that cause disordered sexual development. The vitellogenin over-expression was probably temporarily independent from primary effects on sexual differentiation and likely drove the significant delays to metamorphosis that were observed in all EE2 treatments. Expression analysis of mRNA, as determined by Illumina transcriptome sequencing, is ongoing to help characterize the transcriptional signature of abnormal sexual development and to allow for further assemblage of the molecular pathway that leads to the disordered development.

**EP02C - Androgen-induced kidney hypertrophy in the European bullhead (Cottus sp.): a potential biomarker of androgen exposure**


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The present work was designed to identify an androgeno-regulated signal in the kidney of the European bullhead (Cottus sp.) and to characterise response patterns prior to laboratory and field applications to address androgenic potential of chemicals. In a first step, gender dependent ultrastructural modifications of kidney were quantitated using the Kidney Epithelium Height (KEH) measurement. During breeding period, male bullheads exhibited an increased KEH value of 20.96 ± 3.57 µm compared to females. However, given that genotyping of X. laevis has only recently become possible, studies performed in the past were rarely able to make concrete linkages between genetic and phenotypic sex. Therefore, to further characterize the relationship between genotype and phenotype, X. laevis tadpoles were exposed to 0.1, 0.4 or 1.6 µg/L 17β-ethinylestradiol (EE2), the estrogen analog commonly used in oral contraceptives, from 12 h post-oviposition through 13 wks post-hatch. All EE2 treatments resulted in significant delays in time to metamorphosis. Genotyping showed that genetic sex ratios were similar among treatments. However, morphological evaluation revealed that phenotypic sex ratios were altered in all EE2 treatments. Interestingly, complete male to female phenotypic sex reversal was rare at the concentrations tested, and a surprising number of individuals displayed intersex gonads, abnormal gonads, and atypical vitellogenin over-expression, that were only diagnosed upon histological examination. The impacts of these conditions on fertility and fitness are not known but are likely to be adverse and more complex than complete sex reversal. In all likelihood, the relatively great number of intersex and abnormal animals is a result of estrogens functioning downstream of the initial molecular signals of sexual differentiation. Thus, genetically male animals receive mixed endogenous male and exogenous female signals that cause disordered sexual development. The vitellogenin over-expression was probably temporarily independent from primary effects on sexual differentiation and likely drove the significant delays to metamorphosis that were observed in all EE2 treatments. Expression analysis of mRNA, as determined by Illumina transcriptome sequencing, is ongoing to help characterize the transcriptional signature of abnormal sexual development and to allow for further assemblage of the molecular pathway that leads to the disordered development.

**EP02D - Endocrine disrupting compounds as potential obesogens: musk compounds as a case study**

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Since the discovery of leptin and other adipokines it is now clear that the adipose tissue is not only a storage place for excessive fat but a real endocrine organ, making it possibly sensitive to endocrine disrupting compounds (EDCs). Recently ‘the environmental obesogen hypothesis’ stated that environmental pollutants such as EDCs could
play a role during the development of metabolic diseases such as obesity, broadening the endocrine disrupting concept to a more physiological disruption concept including endocrine, neural and metabolic disruption. This hypothesis could, together with an increased caloric intake, a sedentary lifestyle and genetic predisposition, give potential complementary explanation to the recent epidemic proportions of obese and diabetic patients.

Musk compounds are a group of synthetic personal care products and therefore often daily used. Synthetic musks enter the systemic circulation mainly through dermal absorption and have been detected in human adipose tissue due to their high lipophilicity (Log kow = ± 5). During this study we evaluated the potential obesogenic properties of three musk compounds belonging to the different classes of musks: the nitromusk Musk xylene; the polycyclic musk Tonalid® and the macrocyclic musk Ethylhexyl Brassylate.

The adipogenic potential of the test compounds were evaluated using the 3T3-L1 cell line, a model in vitro cell system for the study of adipogenesis. These cells are fibroblastic and can differentiate into adipocytes after a ten-day exposure with an adipogenic cocktail (isobutylmethylxanthin, dexamethasone and insulin). The Adipored assay, a fluorescent staining for the quantification of lipid droplets associated with the phenotype of mature adipocytes, was used to screen the three musk compounds for their adipogenic potential. Tonalid® was the only compound inducing a dose dependent increase of the lipid droplet formation. The effect of Tonalid® on the differentiation of adipocytes was further confirmed by measuring the gene expression of adipocyte specific marker gene adipogenesis specific protein 2 (ap2) during exposure.

The Adipored assay as well as the expression of the adipocyte specific marker gene ap2 show that Tonalid® is the only musk compound, from the three tested, inducing the differentiation of adipocytes in vitro.

EP02A-5

Development of gene expression biomarkers in cetaceans skin biopsies exposed to bisphenol A (BPA) and perfluorooctanoic acid (PFOA): new tools for emerging contaminants assessment

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Currently, one of the main toxicological issues, in the management and conservation of the marine environment, is the study of the potential impact of compounds released from plastics, such as bisphenol A (BPA) and industrial derivates, such as perfluorooctanoic acid (PFOA). On this regard, the assessment of toxicological risk in wildlife requires the development of sensitive biomarkers including those based on the use of in vitro systems. BPA is one of the most distributed compounds in the world, both in the aquatic and terrestrial ecological systems acting as agonist or antagonist for endocrine receptors. The perfluorinated compounds are used as surfactant and in the surface treatments, they are persistent and it has been shown to act as endocrine disruptors as well. To develop new gene expression biomarkers in cetaceans we exposed skin biopsies of three odontocetes species (sperm whale, killer whale, and bottlenose dolphin) to BPA and PFOA. We selected two potential biomarker genes such as the peroxisome proliferator-activated receptors α and γ (PPAR α and γ). The PPARs belong to a superfamily of ligand-dependent nuclear receptor (PPAR α, β and γ) which regulates physiological processes of lipid homeostasis, inflammation, adipogenesis, reproduction, etc. PPARα and PPARγ seem to be modulated by the presence of BPA and PFOA respectively. The two genes of interest (PPARα, PPARγ) were sequenced in three odontocetes species. The mRNA levels were quantified in response to the two different treatments in the slice samples. Four genes (PPARα, PPARγ, and the previously developed Erx and E2F1) were modulated by the treatments in all the three species. In particular, the results of this set of experiments, revealed that the BPA treatments induce the expression of the genes PPARα and PPARγ showing a dose-response trend. Increasing the BPA concentration increases the bottlenose dolphin, killer whale and sperm whale slices mRNA levels, as well as for E2F1 apart from the killer whale. On the opposite, the PFOA exposure shows a down-regulation of the PPARα and PPARγ both in sperm whale and killer whale slices, while Erx and E2F1 are poorly induced by PFOA in both species. These data represent the first evidence of emerging contaminants effects on cetaceans based on an in vitro experiment and suggest the potential use of this diagnostic markers as early warning signal of exposure to plastic released compounds and emerging contaminants in marine wildlife monitoring.

EP02A-6

Detection of estrogenic and androgenic chemicals in waste waters and peri-urban water bodies in Bulawayo, Zimbabwe

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Endocrine disrupting chemicals (EDCs) as environmental contaminants have received considerable attention since their discovery. In this study, the yeast estrogen and androgen screen tests were adopted to detect the presence and potencies of estrogenic and androgenic endocrine disruptors in treated effluents from sewage treatment plants (STP). Samples were collected from three urban sewage treatment plants in Bulawayo, Zimbabwe. The samples were analysed for mixture ratio design. The selection criteria for inclusion in the mixture was based on estimated exposure (high), antiandrogenic activity (active), registration status (current). The two genes of interest (PPARα, PPARγ), were sequenced in three odontocetes species. The mRNA levels were quantified in response to the two different treatments in the slice samples. Four genes (PPARα, PPARγ, and the previously developed Erx and E2F1) were modulated by the treatments in all the three species. In particular, the results of this set of experiments, revealed that the BPA treatments induce the expression of the genes PPARα and PPARγ showing a dose-response trend. Increasing the BPA concentration increases the bottlenose dolphin, killer whale and sperm whale slices mRNA levels, as well as for E2F1 apart from the killer whale. On the opposite, the PFOA exposure shows a down-regulation of the PPARα and PPARγ both in sperm whale and killer whale slices, while Erx and E2F1 are poorly induced by PFOA in both species. These data represent the first evidence of emerging contaminants effects on cetaceans based on an in vitro experiment and suggest the potential use of this diagnostic markers as early warning signal of exposure to plastic released compounds and emerging contaminants in marine wildlife monitoring.

EP02B-1

Assessment of exposure to estrogenic contaminants in bile extracts of red mullet from Western Mediterranean: an integrated chemical and biological approach using the ER-LUC assay.

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In this work we have identified and measured the concentrations of major metabolites of polycyclic aromatic hydrocarbons (PAHs) and alkylphenols in field collected bile extracts of red mullet (Mullus barbatus) from Mediterranean Spanish waters. Hydroxylated-PAHs (1-naphthol, 9-phenantrol, 9-fluorenol, 1-preneol, 1OH-BaP and 3OH-BaP) and alkylphenols levels (4-n-nonylphenol (NP) and 4-tert-octylphenol (OP)) were quantified in fish bile samples by gas chromatography-mass spectrometry in electron ionization mode (GC-EI-MS). In addition we have applied the estrogen responsive lucerase (ER-LUC) reporter gene assay to measure total estrogenic activity in the same bile extracts of male fish. By integrating the results of both chemical and bio-analytical approaches, we have attempted to explain the measured ER-LUC activity by the calculated potentials based on chemical analysis of hydroxylated PAH and NP metabolites. Our results showed consistent spatial differences in concentrations of hydroxylated-PAHs (2OH-BaP, 3OH-BaP), alkylphenols and estrogenic activity in bile samples, which are presented and discussed.

EP02B-2

Mixtures of the most common antiandrogenic pesticides act additively in vitro: implications for risk assessment

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Evidence suggests that there is a widespread decline in male reproductive health and that mixtures of pesticides may be an important causal factor. At present, 1252 active plant protection products are registered in the US and 411 in Europe, with another 72 “pending”. With such a high number of products on the market, it would be practically impossible to test each substance individually to make robust conclusions about their potential to impact the environment and humans. Therefore, the accurate predictability of mixture effects using modelling approaches is essential for risk assessment. We tested mixtures of pesticides with antiandrogen receptor (AR) antagonist properties using a fixed-mixture ratio design. The selection criteria for inclusion in the mixture was based on estimated exposure (high), antiandrogenic activity (active), registration status (current) and toxicity (less than 10 µM) and led us to select 13 pesticides. We used the MDA-2kb assay to test 3 mixtures: 1. Pure antagonists (8mix: fludioxonil, fenhexamid, ortho-phenylphenol, tebuconazole, dimethomorph, thiamethoxam, pirimiphos-methyl, p,p'-DDE); 2. Dual function agonists/antagonists (5mix: cyprodinil, pyrimethanil, vinclozolin, chlorobutyram, imazalil); 3. All (13mix). Concentration addition (CA) and independent action (IA) models were calculated to predict mixture responses. All the mixtures were within the “prediction window” of CA and IA, whereby CA tended to overestimate, and IA to underestimate, effect. The 8mix agreed with CA, but not IA, whereas the 5mix and 13mix agreed with either CA or IA, depending on inhibition level (10% or 50%) and mixture ratio. For the first time, this shows that multi-component mixtures of widely used pesticides can act together in a predictable and additive manner. Significantly, the mixture test here was composed of some of the most widely used pesticides on foods in Europe and the US. Exposure to pesticides is a complex issue and risk assessment procedure does not incorporate mixture scenarios at present, it is possible that risk to human health and environment is being underestimated, and therefore, effects of mixtures should be taken into account.

Some brominated flame retardants (BFRs) have unintended negative effects on the environment and human health (e.g., endocrine effects). Some of them show a strong bioaccumulation in aquatic and terrestrial food chains, and eos have very persistent, and some show serious toxicological effects such as endocrine disruption [1-5]. During the last few years, a growing number of reports have been published on BFRs. Less toxic alternatives appear to be available already but comprehensive information on their possible toxicological effects and exposure are lacking. The European Commission-funded project ENFINO investigates halogen-free substitution options for some BFRs resulting in a comprehensive dataset on viability of production and application, environmental safety, risk assessment, and life cycle assessment. In total 13 halogen-free flame retardants (HFFRs), consisting of metal-, organic-, and nano-based FRs, as alternatives for decaBDE, TBB-P, and brominated FRs were selected. These were extensively tested and characterized and shows that impurities in some technical HFFR products were responsible for in vitro estrogenic and anti-androgenic activity. The identity of the impurities that were responsible for the endocrine effects could be identified using LC-HRTOF-MS and NMR.

EP02B-4
Investigation of endocrine disruption in Australian aquatic environments - stage 1
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This project uses an integrated approach consisting of multiple in vitro and in vivo bioassays, in situ sampling and trace chemical analysis to compare endocrine disruption at 7 sites in mainland Australia. Sample sites were selected to include a variety of sources such as wastewater discharge, agricultural run-off, industrial effluent, and pristine reference sites. Duplicate 1 L discrete water samples were taken quarterly over a one-year period. Samples were concentrated using solid-phase extraction (SPE) and split into two aliquots, one for in vitro bioassay analysis and the other for chemical analysis. A battery of CALUX assays (estrogen receptor, ER; androgen receptor, AR; progesterone receptor, PR; and glucocorticoid receptor, GR) was used to determine the classes of EDCs present in water extracts. Chemical analysis was used to quantify concentration to identify cause(s) of the observed bioactivity in the water samples. Preliminary results identified at least 11 sites that have estrogenic (or anti-androgenic) EDCs. Analysis with the AR-, PR-, and GR-CALUX assays is currently underway. Chemical analysis has confirmed estrogen mimics (such as bisphenol A, t-octylphenol), the pesticide atrazine, an industrial compound tri(2-carboxyethyl)phosphine (TCEP), and numerous pharmaceuticals and personal care products (e.g., atenolol, dilantin, triclosan, carbamazepine, ibuprofen). Hormone analysis is currently underway. Based on in vitro and chemical data, several stock solutions representative of a selection of exposure concentrations found in the environment will be created for in vivo laboratory fish exposures using a native species (rainbowfish, Melanotaenia fluviatilis) and a widespread exotic pest species (mosquitofish, Gambusia holbrooki) in stage 2. An estrogenic biomarker (vitellogenin) and an androgenic biomarker (in development) will be used to assess whole organism endocrine disruption. The same endpoints will be utilised in situ techniques by sampling fish from polluted aquatic environments identified in stage 1 to assess the amount of endocrine disruption present in the most impacted natural aquatic environments. Finally, in stage 3, a risk assessment will be generated using in vitro, chemical, in vivo, and in situ data to assess the potential risk to aquatic ecosystem health.

EP02B-5
Applying tissue-burden based quality benchmarks to assess the ecological risks of endocrine disrupting organotin compounds in Hong Kong waters
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Since organotins (OTs) in particular tributyltin (TBT) and triphenylin (TPT), have caused widespread adverse effects on marine organisms as the induction of imposex (i.e., superimposition of male sexual characteristics - penis and vas deferens - on females) in over 200 species of neogastropods, and growth inhibition and deformities in oysters. A mandatory global ban on the use of OT-based antifouling systems has been enacted since September 2008. It is, therefore, anticipated that there will be a reduction of OT pollution in marine environments around the world. In this study, we measured the imposex status and tissue concentrations of 11 congeners of TBT and TPT, monoo-PT, di-PT and TPT in the rock shell Tivela clavigera collected from 28 coastal sites of Hong Kong during summer 2010. The results indicated that T. clavigera from a number of sites contained high TPT concentrations and suffered from high degree of imposex. The average TPT tissue concentration was 11,108 μg kg⁻¹ dry weight (dw) in T. clavigera collected from Aberdeen, which was 26 times higher than the maximum TBT tissue concentration in the animals obtained from Kadoorie Bay (i.e., 422 μg kg⁻¹ dw). Using the Monte Carlo approach, an ecological risk assessment was conducted by computing the distribution of risk quotients (RQs); RQ is the ratio between a measured tissue concentration of the target pollutant (MTC) and predicted effects tissue concentration (PETC) (i.e., RQ = MTC/PETC). The results showed that 11.1% of T. clavigera across all sites in Hong Kong waters was at risk with RQ > 1 due to exposure to TPT, whereas the risk associated with TBT was relatively low (0.7% with RQ > 1). Kadoorie Beach, Butterfly Beach, Waterfall Bay and Aberdeen, the four sites which are close to the shipping facilities, were severely impacted by TPT, while all 28 sites were alarmingly heavily contaminated by TPT. As TPT is highly toxic and may trigger imposex in T. clavigera, the local rock shells are still under considerable threat associated with this pollutant even after the global ban of OTs in antifouling systems. This study highlights that TPT is a major environmental concern which deserves immediate actions to control its use and release, and to remediate its pollution in the marine environment of Hong Kong and the Pearl River Estuary.

EP02B-6
Multi-parameter assessment of endocrine disruption in Irish marine waters using biological effects measurements, chemical analysis and passive sampling
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An integrated study examining the uptake and concentrations of selected endocrine disrupting compounds (EDCs) in transplanted Mytilus edulis was carried out at 3 sites on the coast of Ireland. The study combined the biological effects alkali-labile phosphate assay (ALP) and the estrogen luciferase reporter assay (ER LUC), chemical analysis using liquid chromatography tandem mass spectrometry (LCMSMS), and the Polar Organic Chemical Integrative Sampler (POCIS). The exposure was conducted over a three month period with mussels transplanted from a reference site to cages at three coastal locations; Dublin Bay and Galway Bay impacted by secondary treated wastewater effluent and a reference site with little to no anthropogenic input in Galway County on the Irish West coast. Results from the exposure study showed that levels of selected EDCs and their resultant effects in the Irish marine and estuarine waters studied were low. Mussel and water samples were analysed for the estrogenic EDCs estrone, 17β-estradiol, 17α-ethinyl estradiol, nonylphenol, octylphenol and bisphenol A using liquid chromatography/tandem mass spectrometry (LC-MSMS). A similar ratio of the steroid estrogens estrone and 17β-estradiol was detected in water samples and POCIS. The levels detected were in the low ng/L range for water samples, and ng/device for POCIS. The highest concentrations of steroid estrogens were detected in POCIS in July at all sites, with lowest levels observed in September. EDC levels in mussel tissue were detected at low ng/g concentrations. ALP and ER LUC results varied between sites. The natural cycle of seasonal ALP and ER LUC responses in the transplanted mussel stock was used to normalise levels detected in the mussels after transplantation and exposure. Results from this integrated study provide a comprehensive assessment of the levels and potential for biological effects of selected EDCs in Irish marine waters. The suitability of transplanted mussels as biomarkers in environmental monitoring programs is discussed. Transplantation of bio-monitor species, the collection of multi-parameter biological effects and analytical data in addition to collection of data from 'less impacted' areas, when corrected for natural processes, will be vital for the derivation of appropriate future assessment criteria and may be suitable to further support legislative objectives under Marine Strategy Framework and Water Framework Directive monitoring.

EP02C-1
Reproductive responses of two native freshwater fish species populations of Chile, after 5 years of bleached kraft mill effluent discharge and discharge divertoing to sea
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Between 2007 and 2011, we monitored the reproductive status of wild populations of native fish in a basin of central Chile, the potential effect of exposure to effluent discharges from ECF pulp plant cellulose with a modern production and processing system. We demonstrated endocrine disruption at different levels of biological organization in populations of two species of native freshwater fishes of Chile (Percilia gilias and Trichomycterus areolatus) downstream the discharge periods of 2007 to 2009. Depending on the seasonality of monitoring have shown an induction or inhibition of the production of sex steroids, gonadal maturation and histological level increased or decreased during periods of recrudescence and spawning. In January 2010, the plant began to discharge into the sea, but due to the earthquake
of February 27, 2010, the plant discharged into the river again briefly. Despite there was a high variability in the size of the gonad of W. Green1, A. Springer2, N. Saulnier3

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Stably transfected reporter gene In vitro bioassays such as the ER-Luc bioassay (T47D or U2OS cell-based) or the transcriptional activation assay (using HeLa-9903 cells) provide an effect-directed tool for characterizing the estrogenic potency of compounds and sample extracts. A limitation when using these in vitro bioassays for in vivo hazard assessment, however, is that the ADME (absorption, distribution, metabolism and excretion) characteristics of compounds are not taken into account, which can hamper the use of these In vitro bioassays in assessing specific hazard impacts in aquatic organisms. Our aim was to alleviate this limitation by using specific ADME characteristics into account.

We present the work in vitro estrogenicity of a suite of selected (xeno-)estrogenic compounds relative to the reference compound ethinylestradiol (EE2) and intermediates of ethinylestradiol. In vitro estrogenic responses obtained with the uterotrophic assay using subcutaneously exposed rats. The selection of compounds includes alkylphenols, benzo-phenone derivatives, isoflavones, phenyl methanes and steroids. A methodology was applied that calculates EE2 equivalences by not only taking the in vitro derived estrogenic potency of these compounds into account, but also compound-specific, in vitro differences in hepatic availability (the dose escaping hepatic clearance) relative to EE2 as determined by incubations with rat liver microsomes and cytochrome. In addition, compound-specific differences in serum protein binding were assessed using equilibrium dialysis to determine the available concentrations of the selected compounds. The studies demonstrate that combining in vitro estrogenic potency with in vitro determined compound-specific kinetic characteristics for hepatic clearance and protein binding does improve the correlation with the in vivo effect dose obtained with the rat uterotrophic assay, and quantifies the impact of these factors. The improved in vivo predictive value of the in vitro estrogenicity bioassays contributes to the derivation of an in vitro effect-based dose needed for risk assessment of e.g. drinking water samples containing estrogenic compounds. The approach also offers further perspectives for other in vivo predictions for the adjuvant estrogenic potency.
EP03A-2
Stability of functionalized gold nanoparticles in water systems with different composition
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EP03A-3
Application of field-flow-fractionation for the analysis of engineered nanoparticles in complex matrices
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EP03A-4
Quantitative analysis of fullerenes in soil samples
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EP03A-5
Ultrasonic assisted extraction of engineered nanoparticles from environmental samples
M. Farré, M. et al., 2010, First determination of C60 and C70 fullerenes and N-methylfluoropyrrolidone C60 on the suspended material of wastewater effluents by liquid chromatography hybrid quadrupole linear ion trap tandem mass spectrometry

EP03A-6
Radar biofilms and silver nanoparticles: de novo nanoparticle formation and nanoparticle weathering
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EP03B-2
Respiratory distress and biochemical changes in brain but no impact on behaviours of rainbow trout exposed to nanoparticulate titanium dioxide
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Since the advent of nano-ecotoxicology a number of studies, including several from our research group, have demonstrated very subtle biochemical changes in brains of fish exposed to engineered nanoparticles (NPs). This has led us to investigate the implications of these effects for fish behaviour, behaviours link physiological and ecological processes and are key determinants of fish population structure. In this study investigating the effects of TiO2 NPs on locomotor and social behaviour of fish, juvenile rainbow trout (23.7 ± 3.7 g) were exposed to 1 mg/L TiO2 NPs (24.5 ± 10.6 nm, primary particle size) or an equivalent concentration of TiO2 bulk material (134.1 ± 42.5 nm). There is a growing need for risk assessment of different nanomaterials in order to support their safe production and use. For this we need to estimate the exposure concentration of nanomaterials in the environment. We focus on estimating the exposure concentration in water based on removal process such as sedimentation and dissolution. In this study we measured the sedimentation and dissolution of different nanoparticles, PVP capped Ag, Silica coated Ag, CeO2 and C60. In order to cover a broad range of particle properties metal, metaloxide and carbon particles were used. Due to use of HR-ICPMS relatively low concentrations of metal particles could be tested. Furthermore, the results related to properties are combined with the environmental properties of 6 different water types which range in ionic strength, dissoluted organic carbon, acidity and suspended solids. Additionally the effect of the suspended solids is taken into account by comparing sedimentation in filtered and unfiltered water samples. Sedimentation rates and residual concentrations are derived from the observed data. The dissolution measurements show that removal of nanoparticles from the water phase due to dissolution is negligible, except in sea water where up to 10% of added Ag particles dissolved. The removal due to sedimentation ranges from less than 8% to almost 100%. This depends greatly on water characteristics, but large difference are also found between particle types, e.g. PVP vs Silica coated Ag particles. In general high dissolved organic carbon concentration increased stabilization against sedimentation. The description of sedimentation by first order removal kinetics towards a residual concentration seems valid. This is valuable for predicting the exposure concentration of nanoparticles in the aquatic environment.
by interaction of TiO$_2$ NPs at the gill and TiO$_2$ NPs are not bioavailable. Whilst these data indicate that exposure to TiO$_2$ NPs may cause biochemical disturbance in rainbow trout, locomotion was unaffected. Video tracking of movements revealed no significant difference in distance travelled control (195 ± 37 m/h, mean ± SEM, n = 5), bulk TiO$_2$ (167 ± 31 m/h, n = 5), or TiO$_2$ NPs (117 ± 26 m/h, n = 6) exposed fish. Outcome of paired interaction was also not affected. These data suggest TiO$_2$ NPs cause toxicity in rainbow trout but respiratory distress and brain injury exhibited by fish in this study does not impair ecologically relevant behaviours.

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There are currently over 1000 consumer products on the market that contain or utilise nanomaterials, and this number is expected to increase exponentially in the near future in the absence of detailed knowledge concerning the interactions of such materials with biological systems. While nanoparticles have been shown to cross cell membranes, it is not clear to what extent this occurs by particle charge, surface chemistry, or interaction with membrane transport. Furthermore, our inadequate understanding of potential human and ecological effects has resulted in uncertainties of the potential risks of this technology, and impeded the advancement of quantitative risk assessments. The goal this project was to characterize the influence of particle size, shape, and surface chemistry on the movement of gold nanoparticles across mammalian cell membranes, as well as the influence of serum protein concentrations on this uptake. A549 carcinoma human alveolar cells were utilized to characterize the movements of gold nanoparticles across cell membranes. Specifically, the absorption and internalization of three different gold nanoparticle shapes was examined at multiple sizes: spheres (5, 20, and 50 nm), cubes (50 and 75 nm) and rods (20x100 and 20x200 nm). The influence of surface chemistry on absorption was examined through surface modifications of these particles of various shapes and sizes by polyethyleneimine, polyethylene glycol, and citrate. Each particle was characterized by TEM, DLS, UV-vis, and zeta potential. In total, comparisons of cell uptake were examined for 21 different modifications of gold nanoparticles. Also, the influence of serum protein concentrations on this uptake was investigated for nanoparticles with a size of 50 nm and a zeta potential of -30 mV. The results showed that gold nanoparticles can transfer from Chlamydomonas reinhardtii to Daphnia magna via food exposure. This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2011- 0015895).

EP03-4 Trophic transfer of gold nanoparticles in a water food chain W.M. Lee1, S.J. Yoon1, V.J. An2
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The risk of nanomaterials is becoming an issue. Nanoparticles are ultrafine particles and can be easily penetrated through cell membrane. To understand their potential risk, we investigated trophic transfer of nanoparticle in a water-food chain. The low and high trophic level organisms of this study were green alage Chlamydomonas reinhardtii and daphnids Daphnia magna. Chlamydomonas reinhardtii was initially exposed to gold nanoparticles (AuNPs; 10 nm), and 2-day old Daphnia magna were fed by the exposed cells (2.5 [GREEK] 10^5 cell/ml). The results showed that gold nanoparticles can transfer from Chlamydomonas reinhardtii to Daphnia magna via food exposure. This research provides a foundation upon which to predict biological interactions with nanoparticles and facilitate future risk assessment endeavours.

EP03-5 The chronic toxicity of ZnO nanoparticles in Daphnia magna and the application of different detection methods to assess their bioavailability N.C. Adams1, C. Schmitt2, D. Krämer3, E. Company4, J. Galcerán5, R. Blust6
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This research provides a foundation upon which to predict biological interactions with nanoparticles and facilitate future risk assessment endeavours.

EP03-6 Effect of non-ageing and ageing ceria nanoparticles on fresh water micro-algae N. Manier, A. Bado-Nilles, A. Resse, P. Delalain, O. Aguere-Chariol, P. Pandard
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While assessing the hazards properties of nanomaterials in the environment, the main research challenges are numerous. Firstly, determining if nanomaterials are more or less toxic than the bulk forms of the same materials and the extent to which toxicity is governed or influenced by the physico-chemical properties of the nanoparticles. Secondly, it appears necessary to study the effect of nanomaterials and nanoparticles throughout their life cycle including both initial forms and physico-chemically modified form (i.e. aggregated or agglomerated forms) resulting from an ageing process. Our work focused on the effect of commercial ceria nanoparticle (nCeO2) suspensions, towards freshwater micro-algae assessing the effect nCeO2 suspensions with different aggregation/ageing state obtained by using an artificial ageing process. Both ageing and non-ageing nCeO2 suspensions were fully characterized using dynamic light scattering (ZetaSIZER, Malvern Instruments) or laser diffraction (MasterSIZER, Malvern Instruments) and transmission electron microscopy (TEM). In addition, the interaction between the nanoparticles, investigated using flow-cytometry and environmental scanning electron microscope technique (E-SEM). The results obtained showed that the algae growth inhibition was similar after exposure to non-ageing or ageing nCeO2 suspensions. The results obtained from flow-cytometry and E-SEM proved that the ceria NPs are able to tightly entrap the algae cells, which could in part contribute to the effect recorded. Those results also support the fact that aggregation or agglomeration has a few influences when focusing on the standardised algal ecotoxicity test. Moreover by comparison to our previous studies performed with other ceria suspensions, it was shown that the primary particle size and consequently the particle surface area is a relevant parameter in assessing the ecotoxicity of nanoparticles.

EP03-3 Bioavailability of carbon nanotubes to aquatic organisms of different trophic levels and the consequences of CNT-cell interactions to vital functions H.M. Mäes1, S. Rhiem2, S. Hanner3, B. Daniels4, B. Deutschmann4, A. Simon4, S. Giefers4, M. Riding5, K.T. Semple2, K.J. Jones2, E.L. Martin5, W. Baumgartner6, H. Hollert1, A. Schaffer7
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5Multi-walled carbon nanotubes (CNT) were synthesized using 14C-labeled graphene in order to quantify bioaccumulation of this nanomaterial in different aquatic organisms. Next to uptake and elimination, transfer of CNT along the food chain, the influence of the presence of sediment or dissolved organic carbon (DOC) on its bioavailability, and distribution of incorporated CNT to different fish tissues were investigated. Radioactivity was detected in algae, daphnids, blackworms, and zebrafish, which had been exposed to dispersed 14C-CNT material via the water (1 mg/L). Furthermore, single tubes and small agglomerates were visualised by means of transmission electron microscopy (TEM) and the interaction of gold-epithelial cells of daphnids, worms, and ichthyifold of CNT from the fish gills to the blood current was confirmed by the presence of radioactivity in blood samples and gonads. However, CNT were not detected in the brain of zebrafish, which indicates that they do not pass the blood-brain barrier. CNT were not bioavailable for blackworms when spiked to the sediment. Similarly, ingestion of CNT-containing prey by daphnids (CNT-spiked algae) or by zebrafish (CNT-spiked worms) resulted in lower accumulation in the predator compared to uptake of equal amounts via the water phase. Although DOC was shown to keep prepared dispersions more stable over time, its presence had no influence on CNT bioavailability. The consequences of the observed interactions of CNT with cells, following water exposure of different organisms, regarding possible damage to vital functions are investigated at the moment. By now, CNT-associated alterations in the cell
biochemistry of exposed algae were observed by attenuated total reflection Fourier-transform infrared spectroscopy. Furthermore, the condition factor of fish that had taken up CNT during four days, and were daily fed for six days afterwards, was significantly lower compared to the one of control organisms. This might indicate that digestion of food is hindered by the observed presence of CNT material in gut epithelial cells. Longer tests with repeated exposure periods are currently performed to verify this finding.

The combined results of the present fate and effect studies deliver conclusive insights that can be used to assess the possible risks of CNT release to the aquatic environment.

**EP03C-2**

**Short-term toxicity of silver nanoparticles on litter-associated fungi and bacteria from streams**

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Current knowledge of AgNP toxicity is mainly based on laboratory studies with single species, which might not reflect their effects in natural environments. Therefore, it is important to study AgNP toxicity by applying approaches that take the ecological complexity and variability of natural communities and ecosystems into account. Litter-associated fungi and bacteria provide suitable model systems for community ecotoxicology because they play a fundamental role in the various functioning of different ecosystems.

Zinc concentrations in the soil pore water ranged from 1.85 to 12.6 mg Zn/l in freshly spiked soil with ZnO-NP and this increase was found to be linear. Porewater concentrations after three, six and twelve months increased in a non-linear manner with increasing soil concentrations for ZnO-NP and bulk ZnO. Zn concentrations in the pore water, collected from aged soils with ZnCl2, increased with exposure concentration, but also with time.

The release of Zn ions from ZnO-NP was reduced at high spiking concentrations. No effect on Collembolan survival or reproduction was found in three, six or twelve months aged soil material. The toxicity of AgNP in comparison to Ag+ was determined in inhibition tests based on a broad set of functional parameters. The general stress marker heat shock protein 70 (HSP70) and the cytokine-like coelomic cytolytic factor 1 (CCF-1) were differently expressed genes (DEG) decreased with increasing concentration for CuCl2 exposure, whereas for Cu-NP, the number did not change. The number of common differently expressed genes (DEG) decreased with increasing concentration. Differences were mainly related to transcripts involved in energy metabolism (e.g., monosaccharide transporting ATPase, NADH dehydrogenase subunit 1, cytochrome c). Overall, our results indicated that Cu-salt and Cu-NP exposure induced different gene responses. All results pointed to a
nanoparticle-specific effect, and not due to Cu-ions released from the materials, as also indicated by the ISE measurements.

EP03C-6 Long-term effects of sewage sludge spiked with Ag-NP on soil microorganisms
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Due to their antibacterial and antimicrobial properties silver nanoparticles (Ag-NP) are widely used, e.g., in textiles, medical applications or cleaning products. This means that Ag-NP will inevitably enter wastewater treatment plants (WWTP). In the literature, research is reported on the fate and behavior of Ag-NP in WWTPs. Burkhardt et al. (2010) determined that nearly 95% of silver is bound to the sewage sludge whereas around 5% leaves the WWTPs with the effluent in ecologically negligible concentrations.

In the sewage sludge silver ions are transformed to silver sulphide which precipitates. In Germany and other countries sewage sludge is used as a fertilizer in agriculture. Therefore, one of the goals of the present project was to determine long-term effects of sewage sludge spiked with Ag-NP on soil microorganisms over a period of 180 days. We used sludge from a local WWTP and spiked it with silver nanoparticles - NM-300K from the OECD Sponsorship Programme - and silver nitrate. The test soil was the medium acidic and slightly humic loamy sand “Refesoil 01A”. Silver concentrations of 1.6 and 3.4 mg/kg d.m. soil for NM-300K and 2.1 and 4.0 mg/kg d.m. soil for silver nitrate were obtained. We tested potential effects on soil microorganism standardized test systems were used. Ammonium oxidation (ISO 15685) and carbon dioxide production (COTC OECD 217) were performed analyzing samples at day 32, 60, 100 and 180. Two replicate batches were used for each concentration. A control without sludge and one with non-spiked sludge served as reference. Effects on ammonium oxidation or respiration (CTT) of the microorganisms based on Ag-NP or silver nitrate were not observed after 32 days. Referring to the control with sludge we obtained an inhibition of the microbial respiration activity for the highest concentration of silver nitrate after 60 days, whereas ammonium oxidation showed no effect. After 100 days both concentrations of NM-300K and silver nitrate resulted in a comparable respective inhibition of around 30%. The ammonium oxidation test showed a statistically significant dose-dependent inhibition for NM-300K and silver nitrate. Results for day 180 are not yet available. Our results indicate that Ag-NP and silver nitrate can become bioavailable after the degradation of sewage sludge and can cause an inhibition of the soil microorganisms.

EP03D-2 An approach to determine appropriate dose metrics for nanomaterials
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Exposure limits for chemicals are traditionally derived from studies in which increasing doses of the substance are administered. For soluble substances, a unique measure of the dose is the total mass of the substance administered or, equivalently, the total number of molecules administered. Consequently, exposure limits are generally based on mass concentrations, such as mg of chemical substance Y per kg soil. With the advent of nanoscale chemical entities such as nanomaterials, the situation has made its appearance. The different characteristics of nanomaterials (e.g., size, shape, polymorph form of the crystal structure) all may determine their toxic potential. For example, daily intake of X mg of nanomaterial (NM) with particle size d1 may be more toxic than X mg of the same nanomaterial with particle size d2. In other words, for NM information on the administered mass of the chemical substance alone may not be a sufficient description of the dose that determines a particular response. As a result, the question arises of what dose description to use when setting exposure limits for NM. It has been speculated that dose descriptors or exposure limits based on particle number, used for particulate matter, may be more appropriate for NM, while others advocate surface area.

An adequate dose metric for NM should describe all relevant characteristics that are necessary to explain differences between responses. A minimal criterion for an adequate dose metric is that the dose metric should be able to discriminate doses with different response. In its most complete form, the dose of a NM can be described by a distribution function that specifies the number of particles in the ensemble with specific characteristics. For example, PN(d,ξ) may give the number of particles N with a diameter d, surface potential ξ and crystal structure p. Ideally, a dose metric should be as concise as possible, with as few as possible dimensions. A reduced dose metric (for example requiring only information on administered total number of particles, or total surface area) would be pragmatic for risk assessment purposes, since only one exposure limit would have to be derived for various NMs of the same substance. However, a priori there is no reason why such a reduced dose metric should exist. In this contribution, we present a method to determine whether a reduced dose metric for (a class of) NMs exists. As an illustration, the method is applied to results from experiments with various NM published recently.

EP03D-3 Assessment of environmental risks of nanomaterials throughout the product life cycle
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Estimation of exposure due to release into the environment represents a key challenge in determining nanomaterials safety. It is therefore important to understand to what extent existing risk assessment approaches and tools can be applied or will require modification to take account of the particular properties of nanomaterials. The present work aims to identify methodologies and tools to assess and manage the environmental risks due to managed nanomaterials, identify appropriate risk assessment methods, and develop improved methods for risk assessment. Some currently available nano-specific support tools are evaluated, in order to identify gaps where either risk assessment methods and/or input data are insufficient.

A significant problem is that there still remain many outstanding questions regarding the behaviour of nanomaterials in the environment, which could differ from that of materials of similar composition in bulk form. Reducing the level of these uncertainties is essential to proper risk assessment. There is thus an urgent need for reliable data on the environmental behavior and transport properties, toxicity and their transport, persistence and fate, and exposure potential in the environment. However, there is still a lack of basic information on the possible release routes for nanomaterials during production, use and final disposal or recycling. For this reason, it is necessary to improve our present knowledge on the release of nanomaterials from products during all phases of their life cycle. The NanoSustain project is developing innovative solutions for the sustainable use, recycling and final treatment of nanotechnology-based products. Experimental work is carried out to investigate the potential for release of nanomaterials from industrial operations, such as sanding and grinding processes, and during the life cycle of the environment by incineration and recycling by melting. Data obtained from such experiments should help reduce the level of uncertainty in the risk assessment

EP03D-4 A weight of evidence approach for ranking and prioritization of occupational exposure scenarios for engineered nanomaterials
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Nanomaterials are being exploited for a wide variety of military applications. Material science research into the development of new engineered nanoparticles

Engineered nanoparticles are being exploited for a wide variety of military applications. Material science research into the development of new engineered nanoparticles
is far outweighing environmental and human health and safety research, yet the health and safety data are critical for acquisition decisions, regulatory decisions, worker safety, product use and disposal, and public acceptance of nanoparticle-containing products. Traditional life cycle analyses address key steps in nanoparticle synthesis, use, and disposal, but lacks specific information regarding fate and effects in the environment. Conversely, traditional environmental risk assessments address fate and effects of chemicals, but not consider chemicals at specific environmental sites and not throughout the chemical's life cycle. We are developing and applying the comprehensive environmental assessment (CEA) approach, detailed by Davis (2007), which combines life cycle analysis parameters (e.g., manufacture, storage, use, disposal) with traditional risk assessment parameters (e.g., characterization, exposure, effects, assessment) to understand of nanoparticle exposure and effects in different environmental settings. The application of this approach is demonstrated through a case study examining novel nano-based reactive surfaces, a UV-light activated technology. Specifically, we are examining the residue of aged coatings following UV exposure; the most likely release of nanoparticles from this specific nanotechnology. Results of this study represent one of the first documented releases of a nano-sized particulate from a nano-enabled technology. The use for CEA for engineered nanoparticles will improve acquisition, risk, and regulatory decision making and management prior to any unforeseen adverse environment, health, and safety (EHS) events that could dramatically impact the use of these revolutionary new materials.

EP303D-6
Multimedia environmental fate models for engineered nanoparticles - a case study of nano-TiO2 in the Rhine River
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With the increased production and use of engineered nanoparticles (ENPs) their release to the environment becomes inevitable. Multimedia fate models are valuable tools to assess exposure levels of pollutants in different environmental compartments, both at a regional as well as at a global scale and have the potential to be used in a pro-active risk assessment of ENPs. However, whereas multimedia environmental fate models are well established for organic pollutants, the field of environmental fate modelling is still in its infancy for ENPs. Due to the fundamentally different properties of ENPs compared to normal (i.e. low-molecular weight) organic pollutants, it is necessary to completely revise the process descriptions used in the models and to adjust them to account for ENP-specific properties. Here we present a new framework of multimedia fate models to describe the fate and behaviour of ENPs in aqueous environments and predict exposure levels of ENPs. As a first case study and illustration of our new multimedia fate modelling concept for ENPs we present a study on TiO2 nanoparticles (NPs) in the Rhine River. The core of this model is the parameterization of ENP-specific processes. A key process governing the environmental fate of ENPs in aqueous environments is the heteroaggregation of ENPs with naturally occurring suspended particulate matter (SPM) in the nano- and micrometre size range. Deposition of free ENPs by gravitational settling and of ENPs attached to SPM determine the distribution of ENPs between water and sediment compartments. Our model enables the prediction of steady-state concentrations of TiO2 NPs along the course of the Rhine River. Varying the parameters affecting the heteroaggregation process allows us to compare their relative importance on the overall fate and transport of TiO2 NPs in the model system. Overall, the sediment compartment always represents the main reservoir of TiO2 NPs, but the concentration profile and transport potential of TiO2 NPs in the water compartment strongly depends on the heteroaggregation attachment efficiency, R_het-agg, and the characteristics (size, density and concentration) of the SPM.

EP04-1
Green nanotechnology challenges and opportunities
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Nanotechnology is an emerging field, for which there is an unusual opportunity to use science, engineering and policy knowledge to design novel products that are benign as possible to human and environmental health. Recognition of this opportunity has led to the development of the “green nanoscience” concept. Developing an Action Agenda Green nanotechnology has been making great forward progress, but the challenges it has encountered point to an agenda of actions where involvement by the scientific, research and government communities. But there is a pathway forward, and concrete actions that could construct a solid foundation for a profitable and environmentally sustainable future for nanotechnology.

EP04-2
Rapid in vivo assessment of the nano/bio interface to guide safer nanomaterial design
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The rapid rate of discovery and development in the nanotechnology field will undoubtedly increase both human and environmental exposures to engineered nanomaterials. Whether these exposures pose a significant risk remains uncertain. Despite recent collective progress there remain gaps in our understanding of the nanomaterials physiochemical properties that drive or dictate biological responses. The development and implementation of rapid relevant and efficient testing strategies to assess these emerging materials prior to large-scale exposures could help advance this exciting field. I will present a powerful approach that utilizes a dynamic in vivo zebrafish embryonic assay to rapidly define the biological responses to nanomaterial exposures. Early developmental life stages are often uniquely sensitive to environmental insults, due in part to the enormous changes in cellular differentiation, proliferation and migration required to form the required cell types, tissues and organs. Molecular signaling underlies all of these processes. More toxic responses result from disruption of proper motor or sensory signals, thus, early responses result life stages are perhaps the ideal life stage to determine if nanomaterials perturb normal biological pathways. Through automation and rapid throughput approaches, a systematic and iterative strategy has been deployed to help elucidate the nanomaterials properties that drive biological responses.

EP04-3
Ecotoxicology in nanoremediation: n-TiO2 nanoparticles increase Cd bioaccumulation and toxicity in Mediterranean mussel Mytilus galloprovincialis
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The present study investigates the interaction of TiO2 NP with Cd and how the presence of n-TiO2 might affect Cd bioavailability and toxicity in a model marine species, the Mediterranean mussel Mytilus galloprovincialis. n-TiO2 is an efficent photocatalyst largely used in several technological applications including water and aqueous wastes remediation. Cd is one of the most toxic metals of interest for remediation. Both compounds have been quite extensively studied in terms of toxicity in the Mediterranean mussel showing distinct pathways of cellular interaction and biological targets. Mussels have been in vivo exposed for 24h and 7 days to n-TiO2 (1mg/L) and Cd (0.1mg/L) in situ and co-incubation. The documented adsorption of Cd onto n-TiO2 has been confirmed also in our study; the presence of n-TiO2 enhanced Cd bioaccumulation in mussels’ whole soft tissue and increase Cd citotoxicity (reduction of Neutral red retention time in hemocytes). A significant disruption/reversal of Cd capacity to increase the efflux functionality of ABC transport proteins (most probably involved in Cd-resistance) has been also observed in presence of n-TiO2. Our results indicate that specific NP application for remediation purposes might exert indirect toxic effects to the biota by affecting both bioavailability and toxicity of the removed toxic contaminant. The present study will promote nano-ecotoxicology as a new strategy in order to develop eco-friendly nanomaterials for specific technological application as nanoremediation.

EP04-4
Nanoparticle properties affecting embryotoxicity: toward a design of safer nano-Zinc oxide
The pseudo-first-order esterification offers a number of advantages for metal oxide nanoparticle synthesis because it is rapid, produces high yields, permits precise doping, and minimizes waste on the environmental impact of metal oxide nanoparticles is predicted to be increasing. Among metal oxides, nano ZnO (nZnO) is retained one of the most dangerous. Recently nZnO has invaded the market for its UV protective and antibacterial properties, that make it suitable for a wide range of application for functional coating formulations to protect wood, plastics, textiles, paper and artificial surfaces. The vulnerability of ZnO to UV and microbially degradable UV-curing resins data already showed that nZnO has a powerful embryotoxic potential on X. laevis and that it was able to mainly affect gut development. It was clearly demonstrated that nZnO produced severe lesions at the intestinal mucosa and potentially crosses the gut barrier reaching the underlying tissues. In this work we used Xenopus laevis embryos to characterize the embryotoxic and teratogenic potential of nZnO according to the modulation of NP size and surface charge, as well as to the irradiation conditions. To optimize the stability of the NP suspensions and to achieve useful NP concentrations for functional work, we worked in strict connection with a private nanotechnology company with both R&D and commercial activities. The purpose was to provide mechanistic data on nZnO ecotoxicology and to suggest criteria to design safer zinc oxide NPs.

We demonstrated that nZnO-induced embryotoxicity was mediated by NPs' own reactivity rather than ion dissolution and that it is strongly associated with the modality of the biological interactions at the nano-level, which at last depend upon the physical and chemical NP surface properties. These properties are also at the base of the induced oxidative potential by nZnO, which is also very efficiently modulated by light irradiation. Finally NP dimension, and especially surface charge, played a crucial role in determining the embryotoxic potential and the intestinal translocation and lesions of nZnO. The present results showed how a comprehensive knowledge of the nZnO physical and chemical properties, affecting the interactions at the bio-interface, may contribute to make nanotoxicology a predictive science and help chemists and material scientists in the design of safer NPs.

EP04-5
Greener nanomanufacturing: toward low-waste and high-yield synthesis of monodispersed metal oxide nanoparticles
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Metal oxide nanoparticles have attracted much attention due to their wide variety of applications, such as catalysts, energy storage materials, solar cells, light emitting diodes, sensors, transparent electrodes. For practical applications, syntheses should be greener, with low waste and high yields, in order to avoid future environmental health and safety risks and to prevent material resource depletion. If industry plans to use a broad array of metal oxide nanoparticles and employ hundreds of different chemicals to synthesize, we may have to develop a large number of new syntheses and attempt more than hundreds of different toxicological assessments because the specific policies for each environmental impact will be considered including combination of chemicals. To avoid such complicated risks, we require a few generalized synthesis methods of nanostructured metal oxides, technical and environmental performance in order to simplify production and toxicological assessments.

We propose a synthesis route through an pseudo-first-order catalytic esterification using only oleyl alcohol and oleic acid, derived from non-toxic natural oils, as solvents, reagents, and surfactants during a one-step synthesis of metal oxide nanoparticles. This greener approach simplifies the assessment of pollution and toxicity.

We have synthesized metal oxide nanoparticles at 230 °C or less by using an pseudo-first-order catalytic esterification. This approach offers much lower temperature and shorter reaction time than the other methods to produce monodispersed metal oxide nanoparticles with high yield. The produced byproducts are water and oleyl oleate. The oleyl oleate could be recycled to oleyl alcohol and oleic acid again by using hydrolysis. All of metal oxide nanoparticles are monodispersed and show no aggregation. The average diameters of monodispersed In2O3 and ITO nanoparticles are 7.2 nm and 6.3 nm in diameter (with 89 % and 87 % dispersities within ±1 nm differences from the average diameter), respectively. The yield of nanoparticles at 230 °C is greater than 90 %.

The pseudo-first-order esterification offers a number of advantages for metal oxide nanoparticle synthesis because it is rapid, produces high yields, permits precise doping, and minimizes waste. We will demonstrate syntheses of different metal oxide nanoparticles.

EP04-6
Cadmium-free quantum dot nanocrystals for lighting and displays
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Quantum dot (QD) semiconductor nanocrystals have been considered in a broad range of applications, from biological tagging to LEDs, lasers, displays, lighting and solar cells. In their photoluminescent mode of operation, QDs are currently in lighting products, and have the promise to be in liquid crystal display products in the near future. In electronics, quantum dot light emitting devices (QLEDs) are an emerging class of thin-film hybrid organic-inorganic structures that can potentially achieve best-in-class performance amongst large-area emissive light sources [1]. Market research projects world-wide QD production to increase from ~1kg today to ~1000 kg by 2015. Shown in Table 1, the production cost of QDs could further increase to several hundred metric tons.

Historically, QDs are made out a semiconductor core comprising Cadmium Selenide (CdSe). This is due to 1) the early discovery of the QD’s material, 2) the visible wavelength compatibility of this quantum confined system, 3) the general ease of In-VI semiconductor synthesis and 4) the lack of an easily tale and visible-emitting alternative semiconductor. The CdSe system has been ideal for scientific study, and has even led to early product launches most notably in lighting where the environmental net benefit is easily demonstrated. However, due to the combination of known Cadmium toxicity, world-wide regulations, and consumer sentiment, it is desirable to make available an alternative for Cadmium containing QD materials which are Cadmium-free. This green-chemistry guided research and development effort is an ideal example of how nanomaterials afford the opportunity for materials design not just for performance, but simultaneously to minimize the potential for environmental impact. However, when assessing environmental impact, it is crucial to utilize a cradle-to-cradle view of the material’s lifecycle.

EP05 - Non-target analysis and identification of toxicologically significant emerging pollutants

EP05-1
Prioritising non-target identification in wastewater effluent: from picking peaks to programs!
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Non-target analysis of wastewater effluent poses many challenges, including a multitude of peaks and potential interferences of matrix constituents with non-target analysis. However, these samples also offer much valuable information about emerging contaminants in the environment, especially those resulting from urban sources. Here, we detail the results of non-target analysis and prioritisation in several wastewater treatment plant effluents from around Switzerland. The program enviMass was used to perform both target and non-target identification of all samples. The list of non-target masses from all samples were then compared and prioritised according to intensity (and thus, approximately concentration in the environment). This way, thousands of non-target peaks could be reduced to a much smaller list of interesting masses for data-dependant MS/MS analysis to provide fragmentation information for identification of the non-targets. Then, using “the more information the merrier” approach, we gathered as much information as possible from the analysis to hom in on the most likely molecular formulas and thus corresponding candidates. Programs used here included MOLGEN-MSMS (transformation prediction) and Mass Frontier (rule-based fragmentation fragmenter), as well as several different calculations for partitioning and retention properties of compounds. Here, we show how the enviMass workflow allowed a quick and effective selection of peaks of interest and comparison of samples, while the combination of features from many programs improved the chances of non-target identification. While the confirmation of tentatively-identified non-targets is very dependent on the analytical information, the number of possible candidates and the availability, we show how the combination of a few key programs can maximise the use of analytical information and streamline these efforts greatly.

EP05-2
Screening and toxicological evaluation of organic micro-pollutants in the Rhine and Meuse river basins
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The rivers Rhine and Meuse serve as drinking water source for millions of people in Europe. In these waters, rapid improvements in chemical and bioanalytical techniques have led to the discovery of all kinds of emerging contaminants at very low concentrations. Some studies also reported the presence of traces of emerging contaminants in drinking water samples. Dutch drinking water companies therefore intensively investigate their water sources for the presence of emerging contaminants and their fate during treatment processes. Because thousands of compounds are present in the aquatic environment, it is impossible to detect their presence via target analysis in standard monitoring programs. Non-
target screening is therefore used as an additional important tool to obtain information on the types of organic contaminants that are present in the aquatic compartment. It functions not only as an intake monitoring tool, but also as a safety net for new or unknown compounds which are not included in the target analyses.

In this study, sensitive GC-MS non-target screening methods were developed (up to LOD 1 ng/L) and applied to different locations in the Dutch part of the Rhine and Meuse rivers in 2010 and 2011. A first goal of the study was to examine which known and unknown compounds are found in the Rhine and Meuse. By mapping the organic pollution in the river basins, and following the trends of pollution over the year, an integrated picture of the presence of organic pollutants in place and time was obtained. Up to 400 different compounds were found in the water samples. Screening results for the Rhine and Meuse indicated that although there are differences in the occurrence of compounds between the sources, some compounds appear to be widespread (e.g. Surlynol 104 and 5-methyl-1H-benzoazol). Industrial compounds and flame retardants comprise the largest groups of compounds.

A second goal of the study was to investigate whether there are compounds present that are potentially of concern for the drinking water production. To this aim an integrative ranking system was developed in which the identified compounds were sorted according to aspects as their human toxicological risk, frequency of detection, persistence, pathways to the aquatic compartment and associated public concern. A top ten of contaminating compounds that are most relevant for drinking water production from the rivers Rhine and Meuse was compiled. This list will be nominated for political action.

EP05-3
Integrated characterization of a mutagenic waste water treatment plant effluent combining advanced screening techniques and biological assessment
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Increasing numbers of emerging contaminants have been detected in surface water over the last decade. Waste water treatment plant (WWTP) discharges were identified as main sources of these chemicals and related effects in the environment [1, 2]. For this reason, effluents of WWTPs were assessed to gain knowledge about discharged chemicals, their concentrations and their effects on the environment. Linking the discharged chemicals to effects or identifying the substances revealing measured effects is the main challenge for the assessment of WWTP effluents.

To meet this challenge in the present study an integrated biological and chemical approach is applied to identify genotoxic and mutagenic compounds in an effluent of a WWTP treating industrial and municipal wastewater as well as contaminated groundwater. The approach comprises extensive target and suspect screening with biological effects assessment in vitro by Ames fluctuation assay based on a basis of a series of grab samples taken over several weeks and in vivo by fish caging. For chemical characterization of WWTP effluent, a target screening on 300 environmental relevant chemicals and a suspect screening on 1800 chemicals applied or produced at the industrial site was performed. Six site specific suspects were identified. Concentration trends of identified targets and suspects have been focused, focusing particularly on indicator compounds for industrial and domestic wastewater waste, as well as for groundwater co-treated in this WWTP. In the biological assessment, nine of eleven methanotrophic bacteria showed reduced mutagenicity in the Ames fluctuation assay. Based on this data, relationships between target and suspect substances and mutagenicity of samples will be presented.


EP05-4
Identification of biotransformation products (BTPs) formed in freshwater crustaceans
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Freshwater crustaceans, Daphnia magna and Gammarus pulex are widely distributed in watersheds which are contaminated by anthropogenic compounds. After uptake of organic contaminants the organisms possess bio transformation products (BTPs) by means of enzymes involved in detoxification. Little is known on BTPs in crustaceans and their relevance to explain chemical fate and toxicity. In the present study, D. magna and G. pulex were exposed to selected organic contaminants and then their BTPs were determined through the application of target and non-target screening by using high resolution LC-tandem mass spectrometry. The structure elucidation of BTP was performed through MS/MS spectra interpretation with a fragment prediction tool. In addition, the BTP prediction tools currently available were evaluated in terms of the feasibility of application to crustaceans. As the results, various reaction mechanisms such as N-dealkylation, O-dealkylation, N-oxidation, hydroxylation, epoxidation and glycosylation were identified for the BTPs for terrbutryn, terbutryn, tramadol, or venlafaxine. Ingested and terbutryn which both have triazine moiety showed similar biotransformation pathways. No BTPs were identified for valsartan and clarithromycin so far. This may be caused in general by the lower bioaccumulation of the relatively polar and ionized compounds. More BTPs were identified for G. pulex compared to D. magna. A number of dealkylation and oxidation products were successfully predicted by the prediction tools. However, the manual prediction based on biochemical knowledge was most successful.

EP05-5
Input and fate of contaminants in surface waters observed by suspect and nontarget screening with LC-Q-TOF-MS
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Freshwater crustaceans, Daphnia magna and Gammarus pulex are widely distributed in watersheds which are contaminated by anthropogenic compounds. These are increasing numbers of compounds from pharmaceuticals and personal care products, herbicides, fungicides and industrial chemicals. LC with high resolution mass spectrometry enables to perform screening approaches to detect a considerable number of known and unknown analytes in complex samples. In this work we will show the application of target and non-target screening with LC-electrospray ionization-quadrupole-time-of-flight mass spectrometry (LC-ESI-Q-TOF-MS) combined with computer-based data evaluation based on statistical analysis and on tools of computational mass spectrometry to elucidate the input and fate of contaminants in surface water. The software tool MetFrag has been used to retrieve chemical structures of PubChem and to match the measured with in silico fragmentation patterns. A variety of compounds from pesticides, PPCPs and their metabolites could be found. All the number of compounds showed an increasing trend from the source to the receptor. A second objective of the present study was to identify BTPs which could be attributed to the input of the wastewater treatment plant effluents. In the source region the herbicide metabolite desethyl atrazine indicates a historical background of atrazine pollution. Further intermittent findings of amidoizic acid in the source region could be traced back to the influence of storm water overflow. Samples taken downstream of a wastewater treatment plant showed more than 10 000 mass peaks retrieved from the chromatogram by a deconvolution software. Typical wastewater indicators have been found like the X-ray contrast media amiodorizic acid, isopropil and isopsomide, or further pharmaceuticals like carbamazepine and dioflencan, but also the artificial sweeteners ascesulame and sucralose. Further candidates were retrieved from the huge mass list by PCA, e.g. to select relevant candidates for wastewater input. Several compounds could be identified by the application of PubChem search and mass fragmentation match using MetFrag. However, there is still a large number of candidates to be identified due to inconsistent data base and fragment match and due to a high number of possible isomers. In conclusion, there remains a more urgent call for the availability of mass spectral libraries with sufficient numbers of entries.

EP05-6
Can bioanalytical tools help us ensure that our water is safe?
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Chemical monitoring provides a quantitative assessment of single organic contaminants in a water sample but cannot account for the presence of non-target compounds such as unknown transformation products and interactions between chemicals. Bioanalytical monitoring is complementary to chemical analysis and provides information on all bioactive micropolllutants in a sample according to potency, i.e., chemicals of higher toxicity will be weighted higher than less toxic chemicals. Cell-based bioassays provide measures of the cumulative effects of chemicals that exhibit the same mode of toxic action, for which the selected bioassays are indicative, and they can give a measure of the cytotoxicity of all chemicals acting together in a water sample. Improved detection of the presence of chemicals in water enhances risk assessment and informs water management options, among them water recycling from impaired sources such as sewage, or stormwater harvesting and reuse. In this presentation the design of a modular battery of bioassays based on toxicological principles will be presented. This bioanalytical test battery was used for monitoring organic micropolllutants across an indirect potable reuse scheme testing sites encompassing the complete water cycle from sewage to drinking water to assess the efficacy of different treatment barriers, including source control, wastewater treatment plant, microfiltration, reverse osmosis, advanced oxidation, natural environment in a reservoir and drinking water treatment plant. The results of the various studies presented here indicate that bioanalytical tools provide valuable additional information to chemical analysis and should be implemented in the future as a monitoring tool.

EP06-1
Estimating emission source strength of four poly- and perfluorinated alkyl substances (PFASs) in Zurich, Switzerland, using a measurement-and-modelling-combined technique
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SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
From the investigated vegetables, PFAS content: Leafy vegetables > potatoes > pulses & legumes > stem vegetables.

In addition, a long-term study provides information on the time-dependent land use and also any possible displacement of the substances from the soil to percolating water. In landfill containing PFC may also lead to contamination of the food chain via percolation water. A lysimeter study is therefore particularly well suited to observe the carry over offered PFAAs are completely bioavailable. The lettuces were exposed to a set of 10 perfluorinated carboxylic acids (PFCAs) and 3 perfluorinated sulphonates (PFSAs) in four experiment and a comparison of a leafy vegetable to a root/bulb vegetable.

Perfluorinated compounds (PFC) can be taken up by crop plants from the soil of contaminated agricultural lands. Soil contamination may take place through irrigation with PFC contaminated water or by fertilization with sewage sludge containing PFC.

Precipitation, e.g. rainfall may result in only a proportion of the PFC reaching the plants since high water soluble PFC may be washed out of the soil. Discharge from a landfill containing PFC may also lead to contamination of the food chain via percolation water. A lyserver study is therefore particularly well suited to oversee the carry over in plants and also any possible displacement of the substances from the soil to percolating water. In addition, a long-term study provides information on the time-dependent uptake and leaching of PFC.

Lysimeter studies were carried out on monolithic soil columns (with a volume of 1.5 m3). The lysimeter soil received a single treatment with an aqueous solution of a technical mixture of PFOA and PFOS at a concentration of 25 mg/kg soil. Over a period of 5 years the harvests of wheat, rye and rape (canola) were tested for the presence of PFC. Grain and straw were measured separately. Once per month the percolation water was also tested for PFC concentration. In addition to PFOA and PFOS, PFBS, PFPeA, PFFnA and PFPa were detected in the plants material and in the percolation water. The concentrations of PFBS were low in plant material as well as in PFOA and PFOS in the first year of testing (2007). This indicates that short-chain PFC are taken up by plants much smaller than those of longer chain lengths. In addition, PFS, PFPeA, PFFnA, PFPdA and PFOA enter the soil more rapidly than PFOS, the latter only becomes measurable in the percolation water after 4 years. Preliminary results from the long-term studies will be presented.

2) Lechner M, Knapp H (2011) Carryover of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) from Soil to Plant and Distribution to the Different Plant Compartments Studied in Cultures of Carrots (Daucus carota ssp. Sativus), Potatoes (Solanum tuberosum), and Cucumbers (Cucumis Sativus). J Agric Food Chem 59(20):11011-11018

EP06-3 Uptake of Perfluoroalkyl acids by hydroponically and field grown Lettuce (Lactuca sativa) and Radish (Raphanus sativus)

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Perfluorinated alkyl acids (PFAAs) are bioaccumulative persistent, organic pollutants (POPs), which can be detected ubiquitously in the environment. PFAAs pose a risk to human health due to accumulation in the food chain. The occurrence of PFAAs in animals, such as fish, birds and mammals including humans is fairly well documented, but little can be found in the literature about crops or plants in general. Also, most studies focus just on the two main compounds perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA).

Humans are possibly exposed to PFAAs through consumption of vegetables and other plant-related food items. The objective of this study is to understand the accumulation process of PFAAs in crops. In a greenhouse experiment lettuce (Lactuca sativa, var. attraction) was grown hydroponically with a spiked nutrient solution to avoid sorption to soil and to make sure the offered PFAAs are completely bioavailable. The lettuces were exposed to a set of 10 perfluorinated carboxylic acids (PFCAs) and 3 perfluorinated sulphonates (PFSAs) in four different concentrations to assess the difference in behaviour between PFAAs and concentration dependencies.

In a field experiment lettuce and radish (Raphanus sativus) were grown in lysimeters in 4 different concentrations of spiked soil to have a comparison to the greenhouse experiment and a comparison of a leafy vegetable to a root/bulb vegetable.

The results of the concentrations in the different parts of the plants show a different pattern than in the greenhouse experiment with higher concentrations in the foliage part for most of the compounds.

EP06-6 Perfluoralkyl substances in raw and processed vegetables and fruits collected in four European countries; PERFOOD

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The human diet is considered a general source contributing to the overall PFAS burden of the human population. Possible exposure pathways include beverages, food in general and migration from food packaging or cookware. In the EU project PERFOOD, standardized selection of food items, sampling procedures and analytical methods as well as evaluation strategies were applied, enabling a unique assessment of the occurrence of PFAAs in European food as well as the identification of major sources of PFAS exposure for food. During the sampling campaign more than 800 raw food items were purchased, homogenized and after pooling analysed in selected laboratories. This presentation will cover the analytical results for vegetables and fruits acquired in Norway, Belgium, Czech Republic and Italy in perspective to other food items consumed regularly.

In general the PFAS levels found were very low, and mostly shortly chained PFASs up to C8-chains were detected. In general, the most PFASs were detected in samples from Belgium and Norway, followed by samples from Italy and Czech Republic. Mainly PFAAs were detected and occasionally some PFSs, in few samples.

In general, vegetables and fruit seem not to be a main contributor to the human exposure of PFAS via food if not harvested close to point sources. However, data from Belgium show that plants consumed as vegetables and fruit in human diet are able to take up a number of PFASs in the edible parts when exposed to them. Results for the processed vegetables and fruit items will be presented in the presentation.

From processing, videogreens, spinach, lettuce, asparagus, fennel and potatoes, showed highest content of PFAS and will be followed up in the second sampling campaign.

Perfluorinated acids in blood serum from first time mothers from Uppsala, Sweden: temporal trends 1996-2010 and serial samples during pregnancy and nursing

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Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). PFOS levels declined on average 9% per year from over 20 ng/g serum in 1996 to below 10 ng/g.
EP06-06
Does exposure to 8:2 FTOH affect lung function?

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Early life exposures to perfluorinated chemicals (PFCs) have been shown to alter lung and immune function in animal models. We exposed pregnant BALBc dams (G1D6) to 100ng of 8:2 FTOH that was coated onto a cardboard enrichment huts which were replaced weekly. Dams continued to be exposed to FTOH in this way and pups were born to this same environment. Upon weaning, 4 female pups were placed into each cage where they continued to be exposed to FTOH is the same way. Control dams and pups received untreated cardboard enrichment huts. One half of the females of each litter received ovalbumin (OVA) IP at days -21 and -4 and intranasal OVA day -3, -2 and -2. Airway resistance (Raw) was measured by flexVent at 10 weeks of age. Methacholine (MCh) was nebulised and administered in incremental concentrations from 3-50 mg/ml . PC200, the concentration of MCh required to increase the airway resistance 3X from baseline airway resistance was calculated. The PC200 for FTOH exposed animals was significantly less compared to controls. This suggests that early life exposures to 8:2 FTOH may be playing a role in increasing airway hyperresponsiveness in the human population.

EP07-01
Plastics: an emerging risk to the marine environment

Microplastics in the marine environment: synthesis and next steps

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Marine debris is a global problem along coastlines as well as urban, coastal, and offshore waters. Plastic marine debris has been found in surface waters since the 1970s, and an increasing number of observations report plastic debris in the most remote areas of the global oceans. This global long-range transport is possible due to the shared physical properties of many plastic polymers which make them resistant to degradation in the environment. Weathering can slowly break large plastic products into pieces, called microplastics, but full mineralization takes much longer and microplastic particles are expected to persist in the environment for decades. Among many potential risks to the marine environment, microplastics can physically block gastrointestinal tracts of organisms that accidentally ingest these particles, and can serve as vectors of chemicals into natural and artificial environments. This presentation will synthesise known environmental concentrations of microplastics using both historical reports and in original data from the Chesapeake Bay, USA; present the current state of the science regarding the impacts of microplastics; and discuss future steps for this emerging field of research.

EP07-02
UK marine litter monitoring

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The distribution of marine litter on the UK seafloor, including different types and quantities, were mapped by collecting additional data on the back of existing research cruises. Several surveys using specific types of trawls gathered detailed information over a total time period of 19 years (1999 until 2011). The fieldwork experiences allowed us to develop a standard sampling procedure with easy to use data sheets, which facilitates further analysis and future harmonisation across surveys worldwide. The results suggest widespread distribution of marine litter on the seabed of the North Sea, dominated by plastics. The data shows detailed distribution and accumulation patterns in North European waters. There is a considerable variation in geographical abundance between stations, ranging from 0 to 3224 items of debris per km2. Plastic (mainly bags and bottles, 30%) accounted for a very high percentage, more than 70%. Remarkably, the available trend data indicates that quantities of macro marine litter remained relatively stable over the past two decades. In order to investigate this further, the benthic surveys were reinforced with a one off UK case study on marine litter in the water column (2011). High quantities of microplastics were found near major river outlets (>100000 particles/km2). The results will be used to inform policy makers when designing programmes of measures. This analysis is a first valuable contribution to assess marine litter in North European waters and may eventually be used to determine Good Environmental Status (GES) as defined in the EU Marine Strategy Framework Directive, Descriptor 10.

EP07-03
Microplastics in the North Sea region: what kind of quantities and associated ecological effects are we looking at?

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The information available on the composition and distribution of microplastics (≤5 mm) in the North Sea region is rather scarce because the majority of surveys to date have focused on macro-sized plastic litter. Microplastic effects studies are still in their infancy. While society broadly agrees that plastic simply doesn’t belong in the marine environment, regulators are understandably calling for answers to the question, what kind of damage are microplastics doing? Inception studies dedicated to marine microplastics effects need more time to produce a robust body of results. Therefore in this paper, we combine the current scientific knowledge about the amounts and distribution of microplastics in the North Sea region sediments, water and biota with particle toxicity knowledge of these materials from other fields of study in order to estimate the risk of microplastics in the North Sea to the individual organisms and humans exposed to them. By combining data from diverse fields of study (oceanography, ecology, toxicology, drug delivery science, marine biology, etc.) we conclude that field and laboratory evidence for internal exposure in biota gives us an early warning that microplastics are likely to be regarded as bioaccumulative materials and prime biofilm substrates. Considering nano and microparticle toxicity and drug delivery data, we deduce that a variety of effects can be expected depending on the size category of the microplastics in question, since the propensity of particles to be sorbed or eliminated via various routes by various tissues is inherently size-dependent. Inflammatory responses (immune system), cellular damage and other possible physiological, ecological or behavioural effects be expected after the introduction of foreign materials into biological tissues via the gastrointestinal or respiratory systems etc. The bouquet of evidence presented in this paper supports the position that synthetic plastic polymers are fundamentally incompatible materials for input into biological cycles.

EP07-04
Plastic loads on coastal shorelines: where do the micro-plastics come from?

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Plastics have been entering the world oceans for over half a century. Beaches and shorelines are commonly sinks for such debris. However, despite growing concerns over debris, there are no consistent actions to remedy these problems, an understanding of the role micro-plastics play in this and the potential impacts these micro-plastics are having, is still in its infancy. With this in mind coastal and harbour beaches with different known macro-plastic loads were sampled within Central Queensland, Australia to determine the proportion of debris in different size categories.

Debris was collected using strandline transects and randomised quadrats (0.5m2). All surface debris was collected down to a depth of 5cm. Samples were then sieved into different size classes and further categorised (eg. Pellet, Fragment).

Results indicate that areas with high macro-plastic loads also have high micro-plastic loads. Conversely, low impact sites had proportionately less micro-plastics than the corresponding macro-plastic loads. The nature of the shoreline (eg. Degree of wind and wave energy and current patterns) appear to influence the composition of plastics on these beaches with energy environments contributing to the localisation of debris rather than these micro-plastics originating from outside the local area. Further data trends will be presented and the potential impacts to biota and implications for management discussed.

EP07-05
Sorption and desorption of persistent organic pollutants from microplastics in the marine environment
Simultaneous analysis of microplastics and associated plastic additives in sediments in seawater alone. The results suggest that DDT and phenanthrene will desorb from plastics faster as a consequence of digestion by marine organisms than for PVC than PE. The desorption of phenanthrene and DDT from plastic was faster in sodium taurocholate than in seawater, but is slower than desorption of organic contaminants from natural sediments. The results suggest that DDT and phenanthrene will desorb from plastics faster as a consequence of digestion by marine organisms than in seawater alone.
lipids than the gut fluids in the 4 hour in vitro digestion. At the PCB homolog level, bioaccumulation of less-chlorinated PCBs was greater for exposures with PP, indicating release to sediment pore water followed by dermal contact may be an important route of exposure. Thus, plastic debris in the environment appears unlikely to contribute significantly to digestive uptake of PCBs to organisms, that is, perhaps, until weathered plastic particles are small enough that kinetic release during the time-frame of digestion is not limiting, or if plastic particles are in a degraded tissue.

EP07B-4 Occurrence of microplastics in mussels (Mytilus edulis) and lugworms (Arenicola marina) collected along the Belgian-French-Dutch coast L. van Cauwenberge, M.B. Vandengebuerle, M. Claessens, C.R. Janssen Ghent University, Ghent, Belgium Laboratory experiments have shown that various marine organs ingest microscopically small particles. Microplastics smaller than 10 μm were even shown to translocate from the gut to the circulatory system of mussels. However, in all these experiments the exposure concentrations (range 1000 – 30,000 mg.kg-1) were much higher than any field concentrations (e.g. 25 μg.kg-1). As such it is difficult to assess the relevance (risks of adverse effects) of these laboratory observations for organisms living in natural marine environments. The aim of this project was to study the presence, and if present, the concentrations of microplastics in two marine species collected in the field: (1) the blue mussel Mytilus edulis and (2) the lugworm Arenicola marina. Biotas (mussels and lugworms), seawater and sediment from the intertidal zone were collected at 6 beaches along the French-Belgian-Dutch coastline, during September-October 2010. Microplastics were extracted and quantified using an image analysis method followed by a sodium iodide (NaI) extraction, in order to separate the lighter plastic particles from the heavier particles. Seawater was filtered and a NaI extraction was performed on the settled particles. Organisms underwent an acid (HNO3) digestion procedure. Quantification of the extracted particulates showed that: at all sampling locations - all tissue and faeces samples contained these particulates. Tissue concentrations for mussels were around 2 particles.gram-1 of tissue. Lugworms contained only slightly higher concentrations of particulates: 3 particles.gram-1 of tissue. As expected, these tissue concentrations are lower compared to the concentrations found in the environment: mussels were found to contain about 100 particles.g-1 wet matter, lugworms about 20 particles.g-1 wet matter. Although the units are clearly different, these data can be used to illustrate the difference between the environmental and tissue concentrations. Sediment also seemed to have a lower concentration of particulates than seawater. This can be explained by the fact that the sampled upper 5 cm of the intertidal zone is a very dynamic system with high perturbation levels, making it difficult for the suspended particles to settle.

The use of these organisms, sampled for the first time, the assessment of the transfer of microplastics from both the water as well as the sediment matrix to marine life.

EP07B-5 Detection of polybrominated diphenyl ethers (PBDEs) in tissue of seabirds ingesting plastics H. Takada1, K. Tanaka2, R. Yamashita2, Y. Watanuki2 1Tokyo University of Agric. & Technol., Tokyo, Japan 2Hokkaido University, Hokkodate, Japan Polybrominated diphenyl ethers (PBDEs) were measured in the abdominal adipose tissue of short-tailed shearwaters, Puffinus tenuirostris, that were accidentally caught during experimental fishing in the North Pacific Ocean. Their ingestion of plastics has been frequently observed. All the examined seabirds (i.e., 7 individuals) contained microplastics in their stomachs with 0.04 g to 0.41 g per individual. PBDEs were detected from all the seabird samples with concentration range of total PBDEs from 0.5 to 5.4 ng.g-1 wet tissue. In one individual, BDE209 was significantly detected. BDE209 was normally not detected in marine fish and, therefore, exposure of BDE209 to seabirds is a unique exposure pathway. The detection of BDE209 on BDE209 from ingested plastics to the seabird tissue. In addition to this individual, two individuals showed sporadic higher concentrations of PBDEs in their tissue. They were dominated by BDE153 and BDE154 and there congeners profiles were different from the other individuals. No significant correlation was observed between tissue PBDE concentrations and the amounts of plastics in their stomachs. The large variabilities in tissue PBDE concentrations may be explained by large variability in PBDE concentrations in plastic fragments. Our measurement of PBDEs in marine plastics showed large fragment-to-fragment variation among the plastics collected from the same sites. These variabilities can be explained by heterogeneity of plastic fragments in terms of contents of additives and residence time in seawater. Heterogenic nature of the ingested fragments may explain the variation of PBDE concentrations among the seabirds and no significant correlation between tissue PBDE concentrations and plastic ingestion. Analyses of PBDEs in ingested plastics and natural prey are necessary. More number of the seabirds with different amounts of ingested plastics, including individuals with no plastic ingestion, should be analysed to further examine the transfer of PBDEs from ingested plastics to seabird tissue.

EP07B-6 Preliminary results on the potential assumption of microplastics by Mediterranean Fin whale: the use of phthalates as a tracer M.C. Fossi, C. Guerzotti, D. Coppolla, C. Panti, M. Giannetti, S. Maltese, L. Marsili, R. Minutoli University of Siena, Siena, Italy Micro debris floating on the Mediterranean Sea have reached 115,000 particles per km2 with a maximum of 892,000 particles. Impacts of microplastics on organisms and the environment are largely unknown. More than 180 species have been documented to absorb plastic debris including planktrophic species. Until now no data are reported on the potential assumption and effects of microplastics on baleen whales. In this paper we explore for the first time the assumption and potential impact of microplastics in the mysticete species Fin whale (Balaenoptera physalus), suggesting the use of phthalates as a potential tracer of microplastics assumption by Fin whale through micro litter and plankton ingestion. The Fin whale, the only resident mysticete in the Mediterranean Sea (concentrated during the summer in the Marine Protected Area (MPA) Pelagos Sanctuary), feeds largely on the planktonic euphasiacean species, with each mouthful can trap about 70,000 liters of water (including the surface feeding activities), could potentially undergo to the potential risk of the ingestion and degradation of microplastics. The project is implemented through three main steps: Phase I - collection and count of microplastics in superficial plankton samples in Pelagos Sanctuary; Phase II - ecotoxicological investigation of phthalate content in superficial plankton samples of Pelagos Sanctuary; Phase III - ecotoxicological investigation of phthalate content in stranded Fin whale specimens collected on the Italian coasts. Among the 23 superficial plankton samples collected in the present study, "microplastic density"[9.67 debris.m-2m-3] was found in the sample collected close to the Porto Santo MPA (Ligurian Sea). High concentration of phthalate MEHP and DEHP have been detected in superficial plankton samples collected in the Pelagos Sanctuary areas, with values approximately four times higher in the samples of the Ligurian Sea than the samples of Sardinian Sea. Regarding chemical harm to Mediterranean Fin whales, related to the potential assumption of plastic derives, the preliminary data of this paper underlines for the first time the presence in the blubber of four stranded Fin whales relevant concentration of MEHP. This data suggest the use of phthalates as a potential tracer of microplastics assumption by Fin whale by micro litter and plankton ingestion.

EP08- What is the current state of the science on the fate, exposure and effects of pharmaceuticals in the environment?

EP08A-1 Environmental progestin concentrations disrupt oogenesis in amphibians A. Berg1, M. Sahholm1, F. Fack2, A. Norder1 1Uppsala University, Uppsala, Sweden 2Umeå University, Umeå, Sweden Progestins (synthetic progesterone) are extensively used in human and veterinary medicine in e.g. contraceptives and in other hormonal therapies. Recent research shows that progestins pose a threat to egg laying in wild fish. Information on the susceptibility of frogs to impacts from environmental progestin concentrations is lacking. The presence of progestins characterize progestagenic effects on the full cycle of oogenesis (egg development) in frogs, and determine female amphibians' susceptibility to reproductive impacts from an environmental progestin. Levonorgestrel is a commonly used progestogen found in sewage treatment plant effluents at concentrations up to 30 ng.L-1. Sexually mature female Xenopus tropicalis were exposed to levonorgestrel via the surrounding water for 28 days (0, 1, 3, 18, 160 or 1240 ng.L-1). Their ovaries were analyzed histologically with respect to frequencies of immature (in early meiotic prophase I), previtellogenic, vitellogenic, mature, and atretic oocytes. Levonorgestrel exposure caused a reduced proportions of oocytes at immature, vitellogenic and mature stages and increased proportions of previtellogenic oocytes in the ovaries, compared with the controls. The lowest tested concentration,1.3 ng.L-1, increased the proportions of previtellogenic oocytes and reduced the proportions of vitellogenic oocytes, indicating inhibited vitellogenesis. The present study shows that progestin concentrations found in the aquatic environment impaired oogenesis in adult frogs. Our results indicate that progestagenic effects on oocyte development include interrupted germ cell progression into meiosis and inhibited vitellogenesis. Considering the crucial role of oogenesis in female fertility our results indicate that progestins in the environment may pose a threat to reproduction in wild amphibian populations at contaminated sites.

EP08A-2 Identification of active synthetic steroid compounds in impacted river downstream from pharmaceutical industry G.V. Creusot1, S. Aïssa1, J.M. Porcher1, H. Budzinski2, M.H. Dévier2, C. Gardia-Parege2 1INERIS, Verneuil en halatte, France 2EPIC-LPTC-UMR 3805, Talence, France The environmental occurrence of emerging pollutants able to disrupt endocrine signaling pathways other than those mediated by estrogen receptors (e.g. corticosteroids, androgens or progestogens) is of recent concern. Recent evidences have suggested that effluents from pharmaceutical industry release drugs into rivers and trigger adverse effect on wildlife. By using in vitro bioassays combined to passive sampling (i.e. Polar organic compound integrative sampler, POCIS), we previously reported the occurrence of estrogenic, glucocorticoid, anti-mineralocorticoid, progestogen compounds and progesterone X receptor (PXR) ligands downstream a pharmaceutical industry release where strong reproductive alteration have been reported in fish. In this study, we report the use of effect directed approach to identify the compounds responsible for these
Activities were measured as mass balance calculation through chemical analyses directed by toxicity profile. Chemical analyses in POCIS crude extract showed the occurrence of high concentrations of dexamethasone, spiranotolactone, 6-methyl-prednisolone (up to 100 µg/g of sorbent) that well explained GR and anti-MR activities (up to 100%). Conversely, other biological activities (i.e. estrogenic, PXR-like) were poorly explained by the detected chemicals. Then, sample RP-HPLC fractionation was carried out to isolate the different activities. It allowed the isolation of estrogenic and PXR-like activities from GR, PR and anti-MR. It also revealed the occurrence of MR agonists that were masked by the strong anti-MR activity in the crude extract. In addition, HPLC calibration showed a good fitting between retention times of detected chemicals and several active fractions (e.g. 6-methyl-prednisolone in F11, dexamethasone in F12). Nevertheless, some active fractions were left unexplained by calibration standards suggesting that many active chemicals remain to be identified. Chemical analyses in these fractions are under investigations using LC-HRMS system and results will be presented later. It was demonstrated (1) the usefulness of MBA approach using pre-directed chemical analyses based on toxicity profile (2) the strength of the fracionation to unravel complex mixture effect and to finely isolate active chemicals. Overall, our results underscore the need to increase knowledge on the effects of corticosteroids and progesterons on aquatic organisms for better risk assessment.

**EP08A-3**

**Mechanism of action of human pharmaceuticals in fish: the Salpa-phenacetin inhibitor dutasteride as case study**

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**Abstract**

Glycoalkaloid-like alkaloids detected in teleost fishes were also determined and compared with mammalian counterparts. Dutasteride, a synthetic hormone used clinically as a 5\(\alpha\)-reductase inhibitor, caused significant adverse effects in the teleost fathead minnow (Pimephales promelas) by inhibiting the activity of both isoforms of 5\(\alpha\)-reductase (5\(\alpha\)-R). The enzyme that converts testosterone into dihydrotestosterone (DHT), despite the general assumption, in fish endocrinology, that DHT is not synthesized in teleosts, or if it is, it has modest or no physiological relevance. To our knowledge, this class of pharmaceuticals (5\(\alpha\)-R inhibitors) has never before been tested on any fish, or any other aquatic species. The experimental work presented here was divided into two phases: Phase I, focused on the target, and Phase II focused on the effects of the drug in fish. The results of the Phase I showed that 5\(\alpha\)-R is evolutionary conserved in the teleost fathead minnow, both 5\(\alpha\)-R1 and 5\(\alpha\)-R2 genes were expressed in the testis, and DHT was detected in fish plasma at concentrations comparable to the human ones. Our results strongly suggest that DHT has a phallicomimetic role in the fathead minnow, and constituted the rationale for testing the effects of dutasteride in this species. Dutasteride caused significant adverse effects in all the in vivo studies performed during the following up to its potential toxicity on fish, including early life stage and short term reproduction studies, and all the tested life stages were sensitive to the inhibition of 5\(\alpha\)-R activity; however, none of the observed adverse effects were significant at concentrations of exposure lower than 32 µL/L indicating that, at present, the potential presence of dutasteride in the environment (PEC=0.03 ng/L) does not represent a risk to wild fish populations.

**EP08A-4**

**Sub-lethal effects induced by the main cocaine metabolite, the benzoylchonogine, on the freshwater bivalve Dreissena polymorpha**

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The increase in global consumption of illicit drugs has caused both social and medical problems, but also the onset of a potential new environmental hazard. It has been established that after human consumption, drugs and/or their metabolites end up in surface waters, after being carried through the sewage system, posing a potential risk for aquatic organisms. Even if many studies have showed the presence of several drugs and metabolites in freshwater in the high ng/L to low µg/L range worldwide, at present many on their potentially harmful effects on non-target organisms is available. The aim of the present study was to investigate the cyto-genotoxic effects induced by the main metabolite of cocaine (BE), on a classical biological model as the freshwater bivalve Dreissena polymorpha. Our goal was reached through the application of a biomarker battery on zebra mussel hemocytes. The raise of genotoxic effects was investigated by the Single Cell Gel Electrophoresis (SCGE) test, the micronucleus test (MN test), which investigated fixed genetic damage. The Neutral Red Retention Assay (NRRA), by evaluating the lysosome membrane stability, was used to assess benzoylchonogine cytotoxicity. In addition, the activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and the phase II detoxifying enzyme glutathione S-transferase (GST) was measured in the cytosolic fraction. A part of this enzyme battery focused on oxidative status and in treated specimens. 14 days exposure to two increasing nominal concentrations of benzoylchonogine (0.5 µg/L and 1 µg/L) comparable to those currently measured in surface and sewage water, respectively, were performed under semi-static conditions. Our results highlighted that benzoylchonogine exposure induced significant (p<0.05) increases of both primary and fixed DNA damage at both the administered concentrations. In addition, since BE significantly (p<0.03) decreased the stability of lysosome membranes, our data also highlighted its cytotoxicity and the possible antidepressant effect on the observed genotoxic effects. Lastly, BE seemed able to induce moderate effects on the activity of antioxidant and detoxifying enzymes, as shown by the notable oxidative status balance of treated bivalves.

**EP08A-5**

**Chronic effects of diclofenac on fish and mussels assed using human diagnostic techniques**

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This study demonstrates that the non-steroidal anti-inflammatory drug diclofenac is thought to be one of the most toxic pharmaceuticals found in the aquatic environment and has been highlighted by the European Environment Agency as being of particular environmental concern. In the current study we have adapted human diagnostic testing techniques for use with environmental samples. Mussels (Mytilus spp.) and rainbow trout (Oncorhynchus mykiss) were exposed to 1 µg/L and 1000 µg/L dexamethasone under semi-static conditions, with homogenised digestive gland supernatant (15,000g) sampled from the mussels after 7 and 14 day exposure and fish blood plasma sampled after 96. Rainbow trout exposed to the environmentally relevant concentration of 1 µg/L diclofenac for 96h showed a significant increase in alanine aminotransferase (ALT) activity. ALT was considered as a marker of liver health. The addition of (10 µg/L) diclofenac to the experimental setting of treated specimens. 14 days exposure to two increasing nominal concentrations of benzoylchonogine (0.5 µg/L and 1 µg/L) comparable to those currently measured in surface and sewage water, respectively, were performed under semi-static conditions. Our results highlighted that benzoylchonogine exposure induced significant (p<0.05) increases of both primary and fixed DNA damage at both the administered concentrations. In addition, since BE significantly (p<0.03) decreased the stability of lysosome membranes, our data also highlighted its cytotoxicity and the possible antidepressant effect on the observed genotoxic effects. Lastly, BE seemed able to induce moderate effects on the activity of antioxidant and detoxifying enzymes, as shown by the notable oxidative status balance of treated bivalves.

**EP08A-6**

**Effects of environmental relevant concentration of pharmaceuticals on the immune system of Lymnaea stagnalis**

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Lymnaea stagnalis is the most important contributor of pharmaceuticals to the environment; these pharmaceuticals do not occur as single contaminants, but as complex mixtures. The immune system could represent a target system of exposure to this class of toxicants, and Lymnaea stagnalis is likely to be exposed to these compounds. Thus, the major prevailing therapeutic classes (neurological, anti-hypertensive, antibiotic, hypolipemic drugs) were selected, representing compounds of critical concern for the aquatic environment. Their toxicity was tested as a mixture of environmental relevant concentration of each therapeutic class, as a global mixture of all selected pharmaceuticals. However, even if many studies have showed that pharmaceuticals coming from the effluent discharge of L. stagnalis were exposed in semistatic conditions to each mixture in triplicates (3 snails per replicate) during 3 days, and to surface water from the St Laurence River at upstream and downstream of the effluent discharge point. Hemolymph was collected and immunological parameters measured. Hemocyte count and viability was monitored, as well as phagocytosis activity, ROS and thiol production. Gene expression of genes involved the immune response (ALT, TLRA, MDX, SOD, Catalase, SeGPx, GSR, NOS and NOxase) was also measured in real-time quantitative PCR.

Both the environmental concentration of pharmaceuticals mixtures, and the effluent-tainted surface waters modulated the immune response at expression and effect levels. All mixtures decreased hemocyte viability and count. Phagocytosis and ROS production was decreased except with the antibiotic mixture which increased them. All mixture increased thiol in vitro production, suggesting an increase of in vivo antioxidant potential. Except for TLRA, gene expression was generally decreased by drug mixtures. Environmental relevant concentration of pharmaceutical mixtures modulate the immune response of L. stagnalis, the neurological mixture being the most potent, and the antibiotic one having opposite effects compared to the global mixture, but similar effects to the effluent. The effluent decreased cell viability, but increased cell count. Phagocytosis, ROS and thiol production were increased. Gene expression was slightly increased and particularly TLRA. The effects of the effluent on the immune system of L. stagnalis can partly be only explained by the presence of some pharmaceuticals, but also by other factors as the bacterial load.
Since 2006 the Federal Environment Agency (UBA), Dessau, Germany, the Federal Environment Agency (UBA), Dessau, Germany The authorization of medicinal products consists of two phases: a pre-market and a post-market surveillance. For all new marketing applications of human medicinal products under EMA legislation requires an assessment of potential risks to the environment in the pre-approval phase. The respective European guideline on environmental risk assessment of human medicinal products came into effect in 2006 (EMEA/CHMP/PWP/444700/2005 and an amending question and answers document in 2010. The environmental risk assessment according to the EMA guideline is a tiered process in which Phase I is an action limit approach only considering environmental exposure. If the predicted environmental concentration in surface waters (PECaw) exceeds the action limit of 0.01 µg/L, an in depth ERA based on studies on environmental fate and effects shall be performed in a Phase II. For estimating a potential environmental risk, the predicted environmental concentration (PEC) is compared to the predicted no effect concentration (PNEC). A PEC/PNEC ratio > 1 indicates an environmental risk. Since 2006 the Federal Environment Agency (UBA) assessed more than 700 marketing authorization applications. The major therapeutic groups assessed so far are antiinfectives, analgesics, psychotropic, cytostatics and hormones. Based on predicted and measured environmental concentrations a potential risk can be identified e.g. for som hormonal and psychotropic substances. The presentation will illustrate the assumptions and uncertainties of the environmental risk assessment according to EMA guideline. Furthermore the legal provisions and the environmental needs are compared and discussed. To ensure a high standard for the environmental safety of medicinal products for human use a monograph system on active drug substances should be established. Only a pre-marketing monograph system on fate and effects data of drug substances in conjunction with an effective monitoring e.g. of the occurrence of active substances in the environment within the post-market surveillance will be able to ensure the environmental safety of human and veterinary medicinal products in use.

Prioritizing cytotoxic drugs present in aquatic systems and their occurrence in the environment

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Cytotoxic drugs are routinely used in chemotherapy to fight cancer; they work by disrupting critical cellular processes, often through a toxic mode of action. The cytostatics are discharged with wastewaters, largely through the excretion of the unmetabolized drug. This study considers the occurrence and behaviour of these chemicals in aquatic systems via prioritization of old and new anti-cancer agents based on their consumption, excretion and environmental fate. Commonly used cytotoxic drugs were listed on guides for the consumption of data from a detailed hospital surveys of clinical use, which included highly common cytostatics, hormones, as well as their reported presence in environmental wastewaters. In addition to that, we report concentrations from WWTP influent, effluent and receiving waters across United Kingdom. Samples were filtered and extracted using a combination of Strata-X and Florisil SPE cartridges in a LC-MS/MS analytical method. Cyclophosphamide, a commonly used cytotoxic drug, was detected at a maximum concentration of 22.7µg/L in wastewater effluent, with the limit of detection ranging between 0.03-0.12µg/L. This short of chemotherapy work will involve the development of a multi-compound analytical method for the shortlisted chemicals based on a current published method for cyclophosphamide. The analytical method will then be applied in the development of a novel passive water sampler for these chemicals, which generally exhibit high solubility and low KOW values. To date, only limited field studies have reported cytotoxic drugs in the aquatic environment and more extensive surveys are required to assess the distribution of these chemicals and the risk posed to aquatic biota and humans, especially in those areas where water is abstracted for potable water supply.

Economic and environmental pressure from antimicrobials and analgesics during an influenza pandemic

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The global public health community has closely monitored the unfolding of the 2009-10 influenza pandemic to best mitigate its impact on society. However, little attention has been given to the impact that our response to a pandemic might have on the environment. Antiviral, antimicrobial and analgesic drugs prescribed to treat influenza and influenza-associated complications can be excreted into wastewater in a biologically-active form. Here we use a global spatially structured epidemic model to simulate the quantities of drugs used during an influenza pandemic. We couple this model to a wastewater and river flow model, to project drug concentrations in wastewater treatment plants (WWTPs) and receiving rivers in the Thames basin in Southern England. Ecotoxicological models of species sensitivity distributions (SSD) for antibiotics (based on distributions of minimum inhibitory concentrations of antibiotics in pathogens), coupled to an assessment of their mixture toxicity, shows that projected concentrations of antibiotics in WWTPs and receiving rivers would not exceed toxicity thresholds in the case of a mild pandemic, as observed with the current H1N1 pandemic. However, at the peak of a moderate or severe pandemic, the mean antibiotic usage could increase by 13% and 252% as compared to inter-pandemic periods, respectively. Nearly one-third of the microbial community in 70% of the WWTPs in the Thames basin are projected to be growth-inhibited during a severe pandemic. A more severe pandemic might thus result in reduced WWTP efficacy, resulting in the release of partly untreated sewage into receiving rivers, leading to eutrophication, fish kill, and contamination of drinking water abstraction points. SSD modeling of the toxicity of antibiotics to aquatic organisms in the Thames catchment shows that pandemic use is not likely to cause acute toxicity, however, a moderate and severe pandemic might lead to analytic concentrations exceeding chronic toxicity thresholds for ibuprofen.

Emission of human antibiotics and antineoplastics into the environment: identification of high risk exposure scenarios in Europe

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A screening tool has been developed for the identification of high risk exposure scenarios for humans and ecosystems due to the emission of human antibiotics and antineoplastics to aquatic systems. The tool is based on country-specific consumption data, and its use results in a relative ranking of exposure scenarios. We used a spatially explicit approach. A data search was conducted on country- and substance-specific characteristics. These were combined with spatial data on the distribution of agglomerations and sewage treatment plants in Europe, and nation specific emissions were estimated. Calculations of the environmental fate of the antibiotics and antineoplastics were performed with the use of European spatial characteristics on a 100'100km grid scale, a spreadsheet-based multimedia fate model, and biotransformation/biotransformation factors. In these fate calculations, special attention was given to the ionizing properties of the substances, especially those which are zwitterionic, i.e. fluoroquinolones and tetracyclines. Behavioral characteristics of four age-based target groups, i.e. infants, children, adults and elderly, were linked with the concentrations in exposure media derived from the fate calculations. Furthermore, we included specific consumption and activity patterns that could potentially cause high risks, e.g. the consumption of locally grown crops. Acute toxicity data from literature and publicly available databases were used for the ranking of the exposure scenarios for both aquatic ecosystems and humans. The study resulted in a set of rankings of aquatic and human exposure scenarios to antibiotics and antineoplastics on a European scale, and the scenarios most likely to cause risks for the aquatic environment or human health were identified. The rankings were incorporated in a set of GIS-maps to visualize spatial variations and can be used to identify worst-case exposure scenarios and to pinpoint those situations that should be given priority in further, more indepth, risk assessment studies. When data were (partially) lacking, worst-case assumptions were made. Consequently, scenarios for which data are lacking are likely to be assigned a higher rank. The outcome therefore gives an indication of the urgency of the existing gaps of knowledge in the assessment of these exposure scenarios.

Antibiotic-induced change of bacterial communities associated with the copepod Nitocra spinipes

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Antibiotic-induced change of bacterial communities associated with the copepod Nitocra spinipes. The antibiotic-induced perturbation of the microbial community associates with reductions in growth-related traits of the copepod only considering environmental exposure. Thus, in addition to providing evidence that the antibiotic-induced perturbation of the microbial community associates with reductions in growth-related traits of the copepod, this study is first to show that a copoeop serves as a host for endosymbiotic Cardinium. Taken together, our results suggest that (1) antimicrobial substances and possibly other stressors can affect symbiont-mediated interactions in copepods and other hosts, and (2) similarly to other arthropod species, reproductive biology of copepods may be affected by Cardinium bacteria.
The studies were based on the OECD Guideline 317 and involved a 21 day uptake phase followed by a 21 day depuration phase. Physiological differences between the algae, and the mollusc, may explain the difference in toxicity. Using this sort of actual marine data will make the creation of P. Geochronology of pharmaceuticals in coastal marine sediments

EP08C-1
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The sensitivity of Ceramium and Skeletonema may be due to mode of action, 10mg/l, with Skeletonema second. The oyster embryo assay showed little sensitivity. The studies were carried out according to standard OECD or ISO protocols where available, or from peer reviewed research papers. Results showed that for the most part Ciprofloxacin was not likely to be a source of concern in the marine environment with little or no toxicity recorded for most of the species. Most sensitive was Ceramium, with an EC50 of 10mg/l, with Skeletonema second. The oyster embryo assay showed little sensitivity. The sensitivity of Ceramium and Skeletonema may be due to mode of action, or availability in the water column. A log P of 2.3 suggests weak but not exclusively lipophilic behaviour. Physiological differences between the algae, and the mollusc, may explain the difference in toxicity. Using this sort of actual marine data will make the creation of Marine Risk Assessments a more meaningful task.

EP08C-2
Sorption and accumulation of pharmaceuticals in wastewater-irrigated fields in the Mezquital Valley, Mexico

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Due to population growth and urbanization, irrigable arable fields are increasingly contaminated with untreated wastewater worldwide. Environmental and health risks, such as potential endpoints on antibiotic resistance, caused by pharmaceutical residues, are profoundly affected by both environmental conditions and chemicals in the soils. The Mezquital Valley north of Mexico City is the largest wastewater irrigation area facing these risks worldwide. We investigated the accumulation and sorption of the antibiotics ciprofloxacin, sulfamethoxazole, trimethoprim, clarithromycin, and the anticonvulsant carbamazepine in soils with different duration of wastewater irrigation (0-100 years). In contrast to our expectation, no accumulation of extractable ciprofloxacin and clarithromycin was detected. However, sulfamethoxazole and carbamazepine concentrations in the soil (sulfamethoxazole 150-55 µg/kg soil (sulfamethoxazole) was reached after approximately 15 years of irrigation. We hypothesized that this increase in concentrations with irrigation time was at least partly caused by a more efficient sorption of these compounds as a consequence of the accumulation of organic matter in irrigated soils. Sorption of sulfamethoxazole to soils irrigated for different periods of time was, however, weaker than sorption to non-irrigated soil. (De)sorption experiments indicate that a saturation of the sorption sites explains reduced sorption in irrigated soils compared to non-irrigated soil. Sorption of ciprofloxacin was always strong irrespective of the soil matter content and the duration of wastewater irrigation. Sorption strongly counteracts an accumulation of extractable ciprofloxacin in soil. Intermediate sorption leads to an accumulation of extractable sulfamethoxazole and carbamazepine in soil until an equilibrium between input with wastewater and dissipation is reached. Historical records of inputs allow an estimation of dissipation rates under field conditions. The saturation of sorption sites for sulfamethoxazole and carbamazepine during wastewater irrigation and interactions of these substances with other compounds contained in the wastewater increase their leaching to groundwater.

EP08C-3
Sorption and leaching behaviour of four emerging pollutants pharmaceuticals in agricultural soil

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Molasses irrigation and irrigation with wastewater of soils can significantly increase the concentration of pharmaceuticals in soil. The hypothesis of this study was to investigate the mobility of some frequently detected wastewater derived pharmaceuticals in soils under laboratory conditions. Four pharmaceuticals were chosen: carbachazepine(CBZ), venlafaxine (VEN) and their respective major human metabolites, 10,11-dihydro-trans,10,11-dihydroxy carbamazepine (DIOL) and O-desmethyl venlafaxine (ODV). They are frequently detected in wastewater at concentrations higher than 1µg/L and they have different Kow and pKa values. The main results were: 1) batch sorption/desorption study showed that VEN and ODV were largely sorbed by soil and their desorption was dependent on the soil cationic exchange capacity. CBZ and DIOL were little sorbed by both soils and their desorption was not due to cationic exchange. The soil with greater CEC showed more affinity for all compounds. 2) leaching study on soil columns confirmed batch experiment with CBZ and DIOL much less in comparison with VEN and ODV. VEN and ODV were only leached with 100 mM Ca2+ solution. No influence was observed with cationic and anionic surfactant solutions. Retardation of leaching was more important for the soil with greater CEC. This work showed a clear influence of soil CEC in sorption/desorption mechanism of ionic pharmaceuticals VEN and ODV. The Kow value by itself would not be able to predict correctly mobility of ionic compounds in soil. Other parameters related with CEC should be taken into account.

EP08C-4
Uptake of Pharmaceuticals into the Terrestrial Environment

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Increasing amounts of pharmaceuticals are being introduced in soils via sewage, irrigation, leaching and atmospheric deposition. The studies were performed using radiolabelled compounds to allow for a lower limit of detection thus enabling environmentally relevant concentrations to be used. E. fetida and soil samples throughout the experiment were extracted and analysed by liquid scintillation counting. Selected samples were also analysed by radioHPLC to assess whether any metabolism had occurred. The results from the study were modelled using OpenModel (v 1.2 Nottingham University) and based on a one compartment toxico kinetic model. For both flucloxacillin and diclofenac there was uptake within the first 6 hours of the experiment. The modelled uptake rate constants (k1) show that flucloxacillin had a higher overall uptake rate than diclofenac at 0.7428 L/d g-1 and 0.1484 L/d g-1 respectively. When E. fetida were transferred to clean soil both compounds were seen to be...
immediately eliminated from the organism, however modelled depuration rates were considerably slower than uptake rates for both pharmaceuticals. By the end of the depuration phase there were still traces of both pharmaceuticals in the earthworms. The modelling results were then used to evaluate existing quantitative structure-activity relationships used in environmental risk assessment.

Biocenocntrol factors were estimated from the input and depuration data of the pharmaceuticals. The diclofenac BCF was 167 and the BCF for fluoxetine was 133. Both of these values are comparable to BCF values estimated from equations used in risk assessment, such as the Technical Guidance Document. This research shows earthworms, *E. fetida*, can accumulate pharmaceuticals if they are present in soils at environmentally relevant concentrations. Work is ongoing to assess the uptake of additional pharmaceuticals into earthworms, as well as to investigate how uptake can be influenced by soil parameters such as change in pH.

**EP08c-5**

**The anti-arrhythmic drug flecainide: environmental detection and conserved mode of action in fish**

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Numerous recent studies have revealed pharmaceutical residues in the influent and effluent of waste water treatment plants and all kinds of surface water. In these studies many pharmaceuticals were detected which are known to be toxic to aquatic organisms. A high throughput unbiased approach has been taken into account most recently. Therefore we have focused in our study on a multi-residue analysis of polar to lipophilic pharmaceuticals from different trophic levels of the aquatic food chain (fish, prey, fish and fish eating birds).

To determine if flecainide was present in the feed material, which are frequently detected as a metabolite, diclofenac was included in the analyses as a less toxic alternative cardiac agent.

**ET01-1**

**Network inference tools in ecotoxicology: application to Daphnia Magna**


**ET01-2**

**Integrative monitoring of wetlands WWTP effluent remediation in the WIPE project**


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Waterharmonica is a Dutch concept of natural filtration in constructed wetlands, applied to wastewater treatment plant (WWTP) effluent. Particles and nutrients are removed by aquatic organisms and reed beds, and the oxygen balance is restored, leading to positive effects on biodiversity. The WIPE project (Waterharmonica Improving Purification Effectiveness) was an extensive study of wetlands artifically constructed to remediate effluent from three wastewater treatment plants in The Netherlands. The main objectives of this project were to study the fate and toxicity of emerging organic pollutants from the WWTP effluents in order to assess if the discharged water meets the criteria of the Water Framework Directive (WFD) for good quality surface water. An extensive integrated monitoring program was applied at four positions each of three constructed wetland systems, from raw effluent to discharged water. This program consisted of passive sampling, chemical analyses, bioassays and stickleback (Gasterosteus aculeatus) gene expression responses and fish ecology.

Several sampling campaigns were carried out to assess the chemical quality with a wide range of organic chemical analyses and a suite of in vivo and in vitro bioassays. Simultaneously, the ecology of the stickleback (survival, growth and reproduction) was studied by exposing the fish in mesocosms to water from four positions of the wetlands. Large variations in WWTP effluent quality were observed over time. At one of the wetlands an increased fish mortality was observed after exposure to the effluent, while a clear growth inhibition was observed in fish exposed in another wetland. No clear effects on reproduction success were observed in the surviving fish. Chemical analyses revealed that bioavailable levels of most organic micropollutants were not reduced, but a decrease in chemical toxic equivalents was observed after passage of the different wetlands compartments. Certain toxic responses (e.g., estrogenic endocrine disruption) also seemed to decrease in the wetlands. The in vitro ER-Luc measurements of XAD extracts seemed to correlate well with gene expression responses in stickleback. Less clear relationships between in vivo biomarkers and in vitro bioassays were observed for other parameters, such as cytochrome P450 1A (CYP1A) induction, thyroid induction and multi xenobiotic resistance (MXR) inhibition. The WIPE dataset is used to design a smarter WFD monitoring strategy for chemical water quality.
ET01-3  
Transcriptional responses of three-spined stickleback (Gasterosteus aculeatus) to wetland effluent remediation in the WIPE project  
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The putative response was an extensive study of wetlands artificially constructed to remediate effluent from three wastewater treatment plants in The Netherlands. As part of this project, that also included microbiological, analytical chemistry and in vivo and in vitro bioassays, sticklebacks were exposed to water from the wetland systems in flow-through mesocosms, or maintained in a control mesocosm. Fish were hatched in the mesocosms and sampled after 1 year, with livers preserved for transcriptomics.  

Based upon the stickleback genome sequence and results of optimization experiments, a 15,000 probe oligonucleotide microarray was designed for stickleback liver. Hepatic genes differentially expressed in response to 106 female and 75 male sticklebacks from 12 mesocosms. In males, 1493 and in females 4290 transcripts were significantly differentially expressed (FDR<0.05), when comparing control and all exposed fish. The majority of genes differentially expressed between males and females were located on chromosome X, previously identified as the stickleback sex chromosome.  

When assessing a large dataset reflecting responses to complex mixed stimuli, such as WWTP effluents, individual biomarkers can be examined but more systematic approaches to data interpretation using prior knowledge can be valuable. As an example of biomarker response, at one sampling site exposure of the three vitellogenin genes and estrogen receptor alpha were elevated in males. Extracts from passive samplers at this position elicited the greatest response in the ER-luc bioassay. Differentially expressed genes were characterised with respect to prior knowledge of their functions and their chemical regulators and disease associations (Comparative Toxicogenomics Database), resulting in hypothetical predicted profiles of chemicals and biological responses at each site. A number of changes correlated with liver weight, including repression of mitotic genes and associations with cholosterols and fatty acids. Responses that were consistent with exposure to pollutants, such as PAHs, that reflected chemical data showing that some PAHs, including pyrene, exceeded PNEC values. Additionally, responses consistent with PCBs, oxidative stressors, endocrine disruptors and pharmaceuticals and personal care products were highlighted. Benefits and limitations of these approaches will be discussed.

ET01-4  
Effects of metals and pesticides in Enchytraeus albidus - transcription, cellular energy allocation, oxidative stress biomarkers and reproduction: a systems biology approach  
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An important challenge in terrestrial ecotoxicology is to unravel the modes of action of toxicants which are known to have a negative impact on ecosystems. Enchytraeus albidus is an important standard species in terrestrial risk assessment. This species is a typical inhabitant of a wide variety of soil types and fulfills important functions. Effects of chemicals in E. albidus have been addressed mainly assessing effects on their survival and reproduction. The present work comprises results of experiments using E. albidus where effects of the metals cadmium and zinc, and the pesticides dimethoate, atrazine and carbendazim were tested. Different concentrations and exposure durations were used as well as various endpoints from different levels of biological organization - gene expression, cellular energy allocation, oxidative stress markers, survival, reproduction and avoidance behaviour. The main goal was a systems toxicology approach where effects should be integrated. In concentrations known to cause a reduction on reproduction, all chemicals induced significant changes in the antioxidant enzyme activities and substrate levels and, in some of the conditions, oxidative damage was observed. In general, cellular energy allocation (CEA) results indicated reduced amount of energy reserves and disturbing of the mitochondrial electron transport system (ETS) by increasing the cellular respiration. This increase in the ETS activity corroborated the transcriptional evidences, where there was an induction of genes coding for proteins involved in the mitochondrial electron transport system. Gene expression analysis further enabled the identification of common mechanisms of toxicity and also key biological processes affected by each compound. The information gathered with gene expression analysis, along with the reduction of CEA and the verified oxidative damage on the membrane cells, can help to explain the decrease on the reproductive output at a later stage. It further indicated evidences of conserved mechanisms across-species. This presentation includes a combination of several studies that build along years and demonstrates the usefulness of integrating endpoints to better understand and predict the mechanisms of action in a broader context.

ET01-5  
High level dynamical models in Mytilus edulis  
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Introduction. High-throughput omics including transcriptomics, proteomics and metabolomics could help in understanding an organism's response to various stresses. The acquisition and processing of such data for non-model species from their natural habitat is still a challenge, but would enable better comprehension of their response to pollution or seasonal changes.  

Mussels, filter feeding sessile organisms living in the sea have been used for environmental monitoring and necessity makes them particularly suitable for studying the effects of chemicals in coastal regions. Omics technologies alone provide large amounts of information that might prove difficult to interpret. Gene Ontology enrichment analysis can identify pathways that could be involved. Building static similarity networks helps further in finding groups of co-regulated genes possibly important in particular response. The strength of dynamical models in their ability to represent temporal changes, whether due to reproduction or chemical exposure, is another advantage.  

Here we present a systems biology approach of developing high level dynamical models of the annual cycle in blue mussel Mytilus edulis using H-NMR metabolomics data. Metabolites with similar temporal patterns were clustered and ordinary differential equation (ODE) based dynamical models were built between cluster profiles, using NIMOQ [1]. This reveals potential time-dependent relationships between the clusters of metabolites.  

Results. A dynamical model representing Mytilus edulis seasonal variation revealed differences between sexes and females. Perturbations of metabolite levels in Southampton identified clusters of metabolites that show feminising temporal patterns.  

Importance. This work applies dynamic modelling approach to high-throughput data for understanding temporal changes in non-model species living in the wild environment.  

References  

ET02 - Advanced statistical methods in quantitative ecotoxicology

ET02-1  
Statistical ecotoxicology - bright lines and dark alleyways  
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Statistical modelling and inference plays a crucial role in ecotoxicology. Indeed, it could be argued that without the guiding principles of proper statistical design and analysis, ecotoxicology would be so severely handicapped as to be rendered ineffective. Of course there is a lot more to ecotoxicology than concentration-response (C-R) experiments and the identification of ‘safe’ concentrations of contaminants in an ecosystem using statistical concepts such as the Species Sensitivity Distribution (SSD). However, given the prominence and importance of statistics as a critical element of the scientific process in ecotoxicological practice it is both timely and necessary to ask “how well are we doing?”  

This paper attempts to answer this question by providing an overview of the ‘State of Statistics’ (SoS) in ecotoxicology. In it, we briefly trace the history of involvement of statisticians and statisticians and examine the development and population of the ecotoxicologists’ statistical ‘toolkit’. We reflect on what has worked well, what has let us down, and opportunities for future development.

ET02-2  
Well past time to stop using NOELs and LOELs  
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In the popular song, Hotel California, by the band the Eagles, “they just can’t kill the beast”. Th-is is exactly our situation - we (and many others) have not yet been able to kill the “beast”that comprises No Observed Effect Levels (NOELs) and Lowest Observed Effect Levels (LOELs). Given that the concentration-response curve is one of
the founding paradigms of environmental toxicology is it absurd that hypothesis testing continues to be used to report toxicity. We submit that NOEL/LOELs do not meet the criteria of reporting concentration/dose response relationships. The only scientifically defensible measure of toxicity is the description of the concentration/dose response curve. If toxicity tests are going to be research tools, then the data analyses must accurately describe toxicity. Since the 1970s the curve has been demonstrated to provide detailed information, including the slope, the ECx values and the error term. Software is available that makes the computation straightforward. Bayesian curve fitting has been developed that allows estimation of a No Effect Concentration. The Bayesian curve fitting also provides credibility limits for the range of the curve. Since it is clear that the use of the curve is the only rational approach, we propose 5 recommendations: 1. Papers that rely on NOEL/LOELs should not be considered for publication in peer-reviewed scientific journals. 2. Curve fitting is the preferred model for concentration/dose response relationships, ideally including confidence intervals and with supporting data. 3. Using concentration/dose data, concentrations should not be used as descriptions of concentration/dose response data. 4. Papers that rely on historic NOEL/LOELs should only do so after every attempt has been made to replace these data derivations with concentration/dose response data. 5. Papers that treat NOEL/LOELs as data for further analysis such as the derivation of SSDs should receive extraordinary scrutiny. Regulatory agencies across the world must also begin a transition to the use of curve fitting to describe exposure-effect relationships. Although it is understood that for many chemicals NOELs/LOELs are the only results from some toxicity tests, the uncertainties in the accurate representation of the concentration-response curves result in unacceptable uncertainty, obviating informed decision-making.

ET02-6
Estimation of critical effect concentrations: when choosing the right error model matters
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Quantitative interpretation of data requires models of some sort. In general, it requires a model for the process that has produced the observed data, as well as a model for the "noise" (the inevitable deviations between the process model and the data). Useful data interpretation requires a "mechanistic" process model, combined with a noise model that is appropriate for the observations. In (eco)toxicology, toxicity testing of compounds plays a central role. To analyse the data from toxicity tests, curve fitting is very popular. In many cases, this represents a situation with a poor model for both the process and the noise. The process model is just a description of the data that helps to interpolate to ECx. For fitting the process model to experimental data, least-squares analysis is by far the most popular approach in this context. However, least squares represents a noise model, which comes with its own assumptions, which are almost always violated in practice. Toxicokinetic-toxicodynamic (TKTD) models are the most useful type of process model for analysing toxicity data. However, because these models fit the effect patterns over time, they require dedicated noise models. For quantal data such as survival, an appropriate model exists (the multinomial distribution). For continuous data such as body size and reproduction, more research is needed. In this contribution I want to raise the discussion about appropriate process and noise models in ecotoxicology, with an emphasis on the latter.

ET02-5
Variability in ecotoxicology: deliberate ignorance or just not getting it
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In the analysis of ecotoxicological datasets we tend to ignore the variability in the parameters being measured, fail to report known variances, fail to manage overall experimental error rates and ignore the intrinsic value of variability in providing insight into biological responses to stressors. This is probably mostly a reflection of the relative youth of our field of science, a determination to find fundamental or simple answers to puzzling and complex issues and a desire to provide conservative numerical containment thresholds that satisfy the requirements of practitioners and regulators. Nonetheless, it does affect the practice of ecotoxicology in real-world situations and influences the use of environmental management.

In this paper we will examine the general lack of control of experiment-wise Type I error rates in multi-species and multi-sample assessments of toxicity, arguably the most common practical application of ecotoxicology in environmental assessment. While this may be a conservative trade-off that increases the statistical power to detect toxicity by an increase in the Type I error rate, actual measurement of statistical power or Type II error rates are rare in ecotoxicology, as is measurement of experiment-wise Type I error, so the value of this trade-off is rarely known. Modern SSD approaches to compound hazards have been developed that address this problem because ecotoxicologists from many studies are combined without consideration of the effect of compounded Type I errors on an overall model; a model from which, at least in the ANZECC/ARMCANZ framework, specific values of protective concentrations or hazardous concentrations are derived with, again, no overt reporting of error of the overall error rate. Furthermore, the test endpoints used as input to the SSD are commonly "standardised" by the use of application factors and acute to chronic ratios that are either arbitrary or derived from a limited number of tests without consideration of their variances.

The net result is that environmental management targets are specified that are based on layers of conservatism, and that have error rates that could be measured and reported, but generally are not. How much this costs society and operators in terms of compliance with overly-conservative guidelines is not known and incalculable. We believe that it is possible to better report and measure these hidden error rates, and that better insight into them may well provide for more cost-effective management.

ET02-4
Statistical evaluation of experimental designs for modelling of concentration-response functions in ecotoxicology
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The adaptation of experimental designs to regression analysis was rarely discussed in ecotoxicology. The use of D-optimal criterion to develop cost-effective designs is possible to better report and measure these hidden error rates, and that better insight into them may well provide for more cost-effective management.

ET02-3
Interpretation of toxicity data requires appropriate models for process and noise
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Interpretation of toxicity data requires appropriate models for process and noise. This is a strong limitation on their applicability for defining standard protocols for ecotoxicological bioassays. In fact the prior knowledge for ecotoxicological bioassays was recently proposed. But the application of such local optimal designs requires a prior choice of the model, and a good prior knowledge of the model parameters. This is a strong limitation on their applicability for defining standard protocols for ecotoxicological bioassays. In fact the prior knowledge of parameters is often approximate and a local optimal design based on wrong parameter values may be bad. Moreover, calculated optimal values for concentrations may be difficult to experimentally reach, or too precisely defined in regard to measurement precision of the concentration of the studied compound. This is in fact the question we want to address in this paper: how influential is the choice of the error model in the quality of conclusions drawn about hazardous concentrations? With three examples extracted from the literature, we will illustrate the consequences of an inappropriate choice on point estimate of critical effect concentrations as well as on their confidence interval.
concentrations are generally defined following a geometric sequence, often of common ratio 2, centered on the expected LC50 value guessed from prior knowledge. The same number of individuals is generally tested for each concentration and for the control group. We quantified the impact of the total number of tested organisms and of the common ratio of the geometric sequence (for a fixed total number of organisms) on the precision of LC50 and LC50 estimations. Results may help ecotoxicologists to choose the more appropriate design and fix the total number of tested organisms, depending both on their prior knowledge about the LC50 value and on their expectation about precision of estimates.

ET03 - Animal alternatives: methods, endpoints and testing strategies

ET03A-1
An updated assessment of the relationship between the Zebrafish embryo toxicity test and the OECD 203 fish toxicity test - what can we say now?

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This study is part of the joint research project “DanTox” funded by the German Federal Ministry of Education and Research (BMBF) and aims at developing eukaryotic test systems to investigate ecotoxicological effects of contaminated sediments. The visualization of specific activation of the Ah-receptor directly in the zebrafish (Danio rerio) embryo via live imaging is one of these biosassays. The measurement of ethoxyresorufin-O-deethylase (EROD) activity is regarded as a highly sensitive indicator of specific planar polycyclic aromatic hydrocarbons (PAHs) and structurally related compounds. CYP-induced fluorescence signals result from the ability of CYP to convert specific compounds into potentially toxic metabolites. Our results demonstrate that the measurement of ethoxyresorufin-O-deethylase (EROD) activity is a sensitive endpoint that can be used to detect effects of PAHs and other structurally related compounds. The use of this endpoint in combination with other endpoints (e.g., phototaxis) can provide a comprehensive assessment of the effects of PAHs on zebrafish embryos.
Looking for estrogen mimics using transgenic tg(cyp19a1b-GFP) zebrafish embryos

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The tg(cyp19a1b-GFP) transgenic zebrafish expresses GFP under the control of the cyp19a1b gene, encoding brain aromatase. This gene has two major characteristics: (i) it is only expressed in radial glial progenitors in the brain and (ii) it is exquisitely sensitive to estrogens. Based on these properties, we demonstrate that natural or synthetic hormones (alone or in binary mixture), including androgens or progestagens, and industrial chemicals induce a concentration-dependent GFP expression in radial glial progenitors. As GFP expression can be quantified by in vivo imaging, this model represents a very powerful tool to screen and characterize compounds potently acting as estrogen mimics either directly or after metabolism by the zebrafish embryo. This study also shows that radial glial cells that act as stem cells are direct targets for a large panel of compounds, calling to more attention regarding the impact of environmental estrogens and/or certain pharmaceuticals on brain development. Altogether these data identify this in vivo bioassay as an interesting alternative to detect estrogen mimics in hazard and risk assessment perspective.

This work is supported by the CNRS, INSERM, MRT, the ANR NEED and the EU programme LIFE CYCLE (European project n° 222719).

Refining the 48h-zebrafish embryo test - a mode of action dependent approach

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Our results demonstrate that endocrine disruptive chemicals exert their effects on seven endocrine organs such as e.g. embryonic gonads. Here we present a phenotypic anchoring of a comprehensive study on sex-specific gene expression analysis accompanied by histological analysis of endocrine disruption in chicken embryo gonads, having the potential for an animal replacing system for endocrine disruptive toxicologic and ecotoxicologic examinations of chemicals.

Chicken embryos were inoculated with different amounts of tributyltin (TBT) and bisphenol-A (BPA). Embryos were incubated and their gonads analyzed histologically 2 d prior to hatching. From identically treated embryos right and left testes and ovaries were separated and genome-wide transcription profiles generated using SuperTag Digital Gene Expression (ST-DGE, SuperSAGE) profiling.

Looking for estrogen mimics using transgenic tg(cyp19a1b-GFP) zebrafish embryos

ET03A-5


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Embryonic and post-embryonic stages of fish represent an attractive model system for high-content and high-throughput transcriptomics analysis. This technology allows the essential development of a number of specific applications in the fields of toxicology, developmental biology and genomics. In this study, we aimed to investigate the extent of differential expression of genes related to neurotoxicity, using DNA microarrays with 44K and 150K spots.

The results suggest that expression levels of a number of specific genes are associated with the exposure to dichloroacetic acid, and might serve as early biomarkers of neurotoxicity. The scientific and practical implications of these findings include the potential for the development of a new diagnostic method for the identification of neurotoxic pollutants, as well as the identification of potential targets for the development of novel therapeutic strategies for the treatment of neurodegenerative disorders.

ET03A-4

Genotoxicity of uranium in embryonic zebrafish cells

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The toxicity of uranium was assessed using the zebrafish embryo (Danio rerio). Zebrafish embryos were exposed to three different concentrations of uranium (U) for 96 h. The effects on gene expression were analyzed using the Agilent 44K DNA microarray.

The results showed that uranium exposure led to significant changes in gene expression, including upregulation of genes involved in cellular stress response and downregulation of genes involved in developmental processes. These findings suggest that uranium may cause genotoxic effects in zebrafish embryos.

ET03A-3
Harmonised databases for soil and sediment assessment. Measurement of soil and sediment properties has been considered to be the mainstay of soil quality assessment. However, soil quality assessment has been heavily dependent on expert judgement, largely based on experience in the field. A framework for the implementation of soil quality assessment has been designed to promote a harmonized approach to soil quality assessment. The framework includes a set of criteria for the selection of soil quality assessment methods, a classification system for soil quality assessment, and a set of guidelines for the interpretation of soil quality assessment results. The framework is designed to support the development of a robust and comprehensive approach to soil quality assessment, which can be applied to a wide range of soil quality assessment applications.
cycling in terrestrial and marine ecosystems and is prominently reflected in correlations between SI and POPs. Firstly, while 013C and POPs correlated significantly in all species, correlations between 013C and POPs were only significant in the marine foraging WTE (-0.449 < r < -0.388). Secondly, in contrast to negatively correlated 013C and POPs (-0.406 < r < -0.377) in WTE, correlations in NG and GE were positive (0.450 < r < 0.697).

Our results show that variable bioaccumulation patterns between marine and terrestrial foraging predatory birds are related to species-specific feeding ecology. Furthermore, although studying nestlings minimum contamination of certain biological factors, we will further investigate how sex and body mass variation may additionally explain bioaccumulation differences.

ET04A-3

Bioaccumulation and biomagnification of POPs in species from the Scheldt estuary (Belgium)
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As persistent organic pollutants (POPs) are spread worldwide over various environmental compartments, they might also be bioavailable to organisms present in the same surroundings. Their chemical properties like stability, lipophilicity and persistence, give them the ability to easily accumulate into fatty tissues. When pollutants are not excreted or metabolized in the body, they can be passed on from prey to predator, resulting in elevated POP concentrations in species at higher trophic levels. As humans are also at the higher trophic levels, raising details about bioaccumulation and biomagnification processes are of high importance for an accurate risk assessment about POPs in edible species.

In this study, POP concentrations in aquatic species collected from the Scheldt estuary are linked with environmental concentrations and the trophic level of the species, to investigate which POPs are bioaccumulated from the environment into a certain species and which are even biomagnified through the estuarine food chain.

Therefore, fish, crabs, mussels, shrimp, snails, worms, plants and sediment were collected in June 2011 from three locations (Terneuzen, Barth, Antwerpen) along the Scheldt estuary by fine fishing, trawl fishing and by hand. In all the biota samples, 33 PCB congeners, 7 PBDEs, DDXs, chlordanes, HCHs and HCB were targeted for analysis. In the sediment samples, also PBDE 209 was measured. To determine the trophic position of the collected species, carbon and nitrogen stable isotope ratios (013C and 015N) were measured.

ET04A-4

Bioaccumulation and biomagnification of POPs in species from the Scheldt estuary (Belgium)
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Bioaccumulation is the accumulation of a substance in the tissues a living organism through any route. This study investigates which substances can be bioaccumulated in the tissues of aquatic organisms present in the Scheldt estuary (Belgium). The aim of this study is to determine the bioaccumulation capability of various POPs in aquatic organisms such as fish, crabs, mussels, shrimps, snails, worms, plants and sediment collected from the Scheldt estuary.

Materials and methods

1. Introduction

To better understand the impact of pesticide contamination on aquatic ecosystems, we need fundamental information on how pesticides enter food webs and interact with organisms at different trophic levels.

2. Materials and methods

We used model ecosystems with cultivated biofilms and snails to quantify the fate and bioavailability of insecticides with different hydrophobicity (carbofuran, lindane, chlorpyrifos) across a DOM concentration gradient (low, medium, high). Pesticide pulses were added at the start of the 72-h experiments. Treatments without tiles and biofilms were run to quantify passive uptake. Snails were allowed to feed for 48 h. This set-up mimics the mechanisms at the base of food webs that act during runoff-events in agricultural streams.

3. Results and discussion

Partitioning to biofilms reduced chlorpyrifos water concentrations by up to 10±1.0% at high-DOM, but was noticeably higher at low-DOM than at medium-DOM. Concentrations in biofilms were 170.0% higher in the presence of biofilms for all DOM-treatments. Pesticide BCF for biofilms were affected by both DOM and pesticide type, due to differences between lindane and chlorpyrifos treatments. BCFs for snails were only affected by the type of pesticide, and nearly 60% higher for both lindane and chlorpyrifos than for carbofuran. Bioaccumulation factors (BAF) between snails and biofilms showed differences across DOM-gradients for lindane and chlorpyrifos. The lack of concentration-dependent effects of DOM on pesticide fate and bioavailability suggests that also DOM-quality affects these endpoints. Although the share of pesticides recovered in biofilms was consistently less than 1%, their concentrations in biofilms were more than 75% (carbofuran) and 382-times (lindane) higher than those in water.

4. Conclusions

The high and efficient sorption of lindane, and chlorpyrifos show that biofilms may act as a sink for pesticide peak concentrations during runoff-events.

- DOM and biofilms in aquatic environments affect the fate of pesticides following runoff-events and their incorporation in aquatic food webs.

ET04A-5

Food web accumulation of cyclic siloxanes in Lake Mjøsa, Norway
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Lake Mjøsa is the largest lake in Norway and a major source of drinking water for the Oslo area. The lake is located in central Norway and covers an area of approximately 320 square kilometers. The lake is part of the Oslofjord and the lake outflow (Lom) into the fjord is the main outlet of the lake. The lake is known for its high biodiversity and is an important spawning ground for fish species such as Atlantic salmon (Salmo salar) and brown trout (Salmo trutta).

The objective of this study was to investigate the food web accumulation of octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexatetrasiloxane (D6) in Lake Mjøsa food web. The lake food web biomagnification of octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexatetrasiloxane (D6) was analysed by trophic magnification factors (TMFs) and compared with TMFs of legacy contaminants such as polychlorinated biphenyls (PCB) and polybrominated diphenyl ether (PBDE). Of the cVMS, only D5 showed significant TMFs with values above 1, implying biomagnification. The food web accumulation of D5 was however, sensitive to species included at the higher trophic levels, thus whole food web TMF differed from TMF when smelt or trout was excluded. For legacy POPs (e.g. PCB-153), the TMFs were insensitive to the food web composition, as was reflected in a better model fit for PCBs than for D5. Nevertheless, the present study documents food web biomagnification of D5 in the Lake Mjøsa food web, from zooplankton (Daphnia and Calanus) and Myiidae to planktivorous (vendace and smelt) and piscivorous (trout) fish.

ET04A-6

Trophic transfer of decamethylcyclopentasiloxane (D5) in aquatic food webs in comparison to polychlorinated biphenyls used as benchmark compounds
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Decamethylcyclopentasiloxane (D5) is a cyclic siloxane that is widely used as a silicone fluid. It is a colorless, odorless, and tasteless liquid that is non-toxic and non-flammable. D5 is used in a variety of applications including cosmetics, pharmaceuticals, and consumer goods. However, the environmental impact of D5 and other cyclic siloxanes is not well understood.

In this study, the trophic transfer of D5 was compared to the trophic transfer of polychlorinated biphenyls (PCBs), which are widely used as benchmark compounds for bioaccumulation. The study was carried out in aquatic ecosystems, where D5 and PCBs were added to the water column and the fate and bioavailability of these compounds were monitored.

The results indicated that trophic dilution of D5, not biomagnification, was occurring across the aquatic food webs, which is the highest level of agreement for results between Lake Pepin and Oslofjord. The study demonstrated that trophic dilution of D5 was not related to differences in the environment (freshwater vs marine) or to exposure to metal bioaccumulation by aquatic invertebrates in the environment can often be explained by chemical parameters such as metal concentrations within the solution and particulate phases by exposure-dose-response relationships. Simple relationships of this type are not found for invertebrates in the highly turbid Strickland River, Papua New Guinea. Here, indigenous prawns demonstrate significant differences in bioaccumulated metal concentrations (predominantly cadmium) between populations exposed to mining effluents compared to those in reference tributaries. However, metal concentrations of waters and sediments are not significantly different between sites. This study investigated the potential sources and mechanisms of cadmium (Cd) bioaccumulation by the freshwater decapod Macrobrachium australiensis using 64Cd-labelled
water and food sources. Synthetic river water (SRW) was spiked with environmentally relevant concentrations of Cd and prawns were exposed for seven days with daily renewal of test solutions. Prawns were subsequently allowed to depurate in Cd-free SRW for fourteen days. Dietary assimilation of Cd was assessed through pulse-chase experiments where prawns were fed 109Cd-labelled fine sediment, filamentous algae and carrion (represented by cephalothorax tissue of water-exposed prawns). Radio-analyses during the exposures were used to determine influx and efflux rate constants for Cd in water, and the assimilation efficiency (AE) and efflux rate (E_r) of Cd from each dietary source.

Results indicated that M. australiense readily uptakes Cd from solution and that uptake rate increased linearly with increasing exposure concentration. During depuration, water efflux rates were low (0.9 ± 5 % d^-1) and were not dependent on exposure concentration. AE of dietary sources were comparable for sediment and algae (approx 50%), but lower for carrion (33 %) and efflux rates were low (0.2-2.6 % d^-1).

The results demonstrated that prawns are likely to bioaccumulate Cd readily from both water and food sources. The rapid uptake but slow efflux of bioaccumulated Cd may explain why monthly or weekly measurements in water and sediments provide inadequate information regarding exposure or dose to explain metal accumulation patterns. A biokinetic model of Cd accumulation by M. australiense is based on the findings.

ETOBD-2
Bioavailability of dissolved organic carbon-complexed 65Cu-DOC

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Stable Cu isotope (Cu/Cu) was complexed with various representative dissolved organic carbon (DOC) types, including coastal seawater DOC, fulvic acid (FA), cyanobacteria secreted products (PPDOC), haptophyte diatom-biogenic calcite (DADC) at different concentrations. The uptake of these dissolved Cu species by the coastal green mussel Perna viridis was quantified for the first time. Copper complexed with different DOC types were taken up in some measure by mussels, depending on the DOC types. However, complication generally reduced Cu uptake as compared to that of inorganic Cu species, and DOC type-specific negative relationships were found between DOC levels and Cu uptake. Strong Cu binding sites (including HA and organic sulfur functional groups) within DOC appeared to control the inhibitory effects of DOC on Cu uptake, possibly due to the competitive binding of Cu between the dissolved phase and biological membranes. Therefore, differences in strong Cu binding sites may explain the differences in bioavailability of Cu complexed with different types of DOC. At the same time, the variations in Cu-DOC uptake may also be partially attributed to the absorption of Cu-DOC complexes, especially for the small Cu-DOC complexes (e.g., Cu-Cys, His or DDT). Our study highlights the importance of considering the species of Cu-DOC complexes when assessing biological exposure to dissolved Cu in natural waters, especially during events, such as phytoplankton bloom periods.

ETOBD-3
A stratified probabilistic survey of mercury in biosentinel fish and Diffusive Gradient in Thinfilm devices in San Francisco Bay, CA, USA

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Although there are many regional programs to monitor contaminants in fish, few employ probabilistic survey designs, with hypothesis testing and comparison across biosentinel types. We surveyed mercury (Hg) concentrations in forage fish to determine spatial patterns in Hg bioaccumulation across 99 sites in San Francisco Bay. We also investigated these results to concentrations in field deployed Diffusive Gradient in Thinfilm devices (DGTs), and determined Hg stable isotopes in fish and sediments, to evaluate potential sources of fish Hg. Results indicated that forage fish Hg and Hg isotopes followed a broad spatial gradient in the Bay. Fish Hg concentrations and 198Hg isotopes significantly declined with distance from the Guadalupe River, which drains historic mining areas. Fish and sediment Hg isotopes were highly correlated (r = 0.91), suggesting sediments derived from mining and other historic sources to be the primary source of Hg in the fish. Fish Hg concentrations were reduced at sites draining waste water and sediments from mining. Furthermore, non-biological parameters were not well correlated, with DGT samples exhibiting high variability among nearby sites, and elevated Hg concentrations near locally contaminated sediments. In combination, these findings indicate that Hg methylation and bioaccumulation in San Francisco Bay involves coupled processes operating on separate spatial scales. Wheras DGTs reflect processes operating at local scales, the ultimate provenance of fish Hg appears to be historic sediments, driven by broader regional gradients.

ETOBD-4
The role of feeding habits in controlling the Hg bioaccumulation and biomagnification in freshwater tilapia

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The bioaccumulation and biomagnification of mercury (Hg), especially methylmercury (MeHg), is commonly found in aquatic organisms. The mercury level in fish is not only related to its surrounding water conditions but also the feeding habit as well, and predatory fish always has high Hg level because of the Hg biomagnification through trophic transfer. Whether the biomagnification potential (in terms of trophic transfer factor, TTF) could be influenced by feeding habit is an interesting but unknown question. To explore this issue, we conducted a 40-days Hg (both inorganic and organic form) accumulation experiments, by feeding freshwater tilapia with three types of Hg labeled food (aquatic grass, freshwater shrimp, commercial pellets) at a certain ingestion rate. The results showed great differences between inorganic Hg and MeHg accumulation patterns. More important, it revealed for the first time that the prey type could greatly affect the accumulation patterns as well as fish growth and the Hg biomagnification potential. The dietary biomagnification and assimilation rates were found to be different using radiotrace technique, and provided a good explanation of the underlying mechanism. Moreover, a three-month field study was designed to investigate the influences of feeding condition to Hg accumulation process in tilapia under local conditions, by comparing the growth and Hg accumulation of fish on feeding on commercial food and natural food. These results again showed the important role of feeding habit in controlling the Hg bioaccumulation and biomagnification process in fish.

ETOBD-5
Dietary accumulation, maternal transfer and metabolic inter-conversion of methoxylated, hydroxylated and synthetic PBDEs

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Polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants, and the structurally related hydroxylated PBDEs (OH-PBDEs) and methoxylated PBDEs (MeO-PBDEs) occur in precipitation, surface water and in wildlife and humans. The occurrence of OH-PBDEs in wildlife and humans is of considerable concern due to their greater toxicities relative to PBDEs and MeO-PBDEs. Although the precise origin of OH-PBDEs has not been determined the dominant hypothesis has been that OH-PBDEs are formed by hydroxylation of PBDEs by phase I enzymes and MeO-PBDEs are then formed by methylation of the OH-PBDEs. The aim of this study was to investigate the hypothesized biotransformation or inter-conversion of PBDEs, MeO-PBDE and OH-PBDE. Specifically, we used 1) an in vitro approach with hepatic microsomes from metabolically activated rats, chicken and rainbow trout to characterize the metabolic relationships among these compounds; and 2) an in vivo approach to study the dietary accumulation, maternal transfer, and tissue distribution of PBDEs, MeO-PBDE and OH-PBDE in sexually mature Japanese medaka (Oryzias latipes). Results obtained both from in vitro and in vivo experiments demonstrated significant production of OH-PBDEs from MeO-PBDEs while methabolization of PBDEs to OH-PBDEs was negligible. It is hypothesized that this previously unidentified mechanism is the main contributors of OH-PBDEs found in wildlife and humans. These results suggest that risk assessment of PBDEs and their metabolites needs reevaluation and human exposure to MeO-PBDEs that occur naturally in marine organisms should be considered.

ETOBD-6
Use of fugacity ratio methodology for assessment of bioaccumulation potential: PCB Congeners versus eVMS materials D4 and D5

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Participants in a 2009 SETAC Pellston Conference concluded that the fugacity ratio approach is a practical framework for decision making in chemical management and regulation, particularly with regard to assessment of bioaccumulation potential. A fugacity ratio expresses the status of chemical distribution relative to a state of chemical equilibrium, which is thermodynamically defined as a situation where the fugacity ratio is 1. A fugacity ratio greater than 1 therefore indicates that the chemical in the organism is able to achieve a higher fugacity (or chemical activity) than that in the medium to which it is exposed. In that case, the organism is able to magnify the chemical potential in its environment. A fugacity ratio less than 1 implies that the chemical concentration in an organism is less than its thermodynamic equilibrium value in the
Partitioning of organic chemicals to structural proteins

It is essential to consider the biotic magnification or biomagnification when assessing the environmental fate of chemicals. This usually occurs by testing whether fugacity ratios are greater than unity (1.0). This approach has been applied to the cyclic volatile methylsiloxanes (cVMS) materials octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), along with positive controls, such as PCB congener 153 (2,2',4,4',5,5'-hexachlorobiphenyl). The laboratory and field studies of D4 and D5 in fish and sediment/soil (BASF). Field data also show fugacity ratios for PCB 153 greater than unity in biota/water, biota/sediment, and in food webs. In comparison, similar laboratory and field fugacity ratios for D5 and D4 were smaller than 1.0.

**ET04C-1**

Evolution of fugacity ratio (FGR) methodology to assess the theoretical potential for biomagnification in aquatic and terrestrial systems and to better understand the factors influencing the magnitude of biomagnification in different biotic environments. The approach is based on the concept that the fugacity ratio (FGR) is a measure of the degree of biomagnification relative to the fugacity of the chemical in the aquatic or terrestrial environment, with values greater than 1 indicating biomagnification. The FGR methodology allows for a more systematic and quantitative assessment of biomagnification processes in different environments, providing insights into the mechanisms and factors controlling biomagnification.

**ET04C-2**

The effect of pH on the toxicity and bioaccumulation of weak organic acids and bases is pH dependent ionization in the biologically relevant pH range, wherefore standard test procedures for these compounds can be applied. The effect of pH on the uptake of octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) is strongly influenced by pH. The pH effect is higher for those compounds with intermediate Kow values. An accurate risk assessment of these compounds is therefore necessary to take the pH of the exposure solution into account. However, toxicity testing and bioaccumulation at multiple pH levels represents a great economic cost - particularly when it is considered that up to 33% of the industrial chemicals registered for REACH are weak acids or bases.

Based on a compilation of available pH dependent toxicity tests using ionizing compounds it is stipulated that a single worst-case test may suffice. For acids this would optimally be two pH units below the pK value of the compound, and 2 units above for bases. For some compounds this will lead to tests outside biologically relevant range, wherefore a minimum of pH 6 and maximum of pH 9 is suggested for these tests. Acids with pK values below 2, and bases with pKa values above 12 do not undergo pH dependent ionization.

**ET04C-3**

Modelling specific mechanisms of bioaccumulation: protein binding and active uptake of surfactants

Chemicals that bioaccumulate by specific mechanisms in addition to lipid/water partitioning reveal characteristic physico-chemical properties. The range of substances showing i.e. active mechanisms of uptake in the gastro intestinal tract (GIT) or specific protein binding include, for example, poly- and perfluorinated carboxylic acids. Data describing processes and symptoms of specific mechanisms of bioaccumulation of surfactants (target chemicals), pharmaceuticals and industrial chemicals (reference chemicals) have been retrieved from the literature. Emphasis was laid on interactions (partition coefficients, rate constants and binding affinities) related to binding to serum proteins and active transport phenomena. LSER modelling provides a mechanistic interpretation of relevant processes.

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**ET04C-4**

Partitioning of organic chemicals to structural proteins

A number of partitioning and binding studies have been reported for blood proteins such as serum albumin. However, there are only limited data available for partitioning of organic chemicals to structural proteins. These proteins are not made to bind chemicals, and thus their binding may be nonspecific, and the extent of the binding may be smaller than that of proteins that serve as a transporter or a receptor. However, relative abundance of structural proteins is generally high in organisms and thus partitioning to the lipid phase may be significant. In this study, binding of various neutral organic chemicals to several model structural proteins is evaluated with experimental and modeling approaches. Proteins from chicken and codfish muscle were similar in terms of binding of neutral organic chemicals. In contrast, binding by collagen was generally weaker than that of the muscle proteins. Binding to bovine serum albumin (BSA) measured before was generally stronger than that of the studied structural proteins. Thus, although serum albumin is often considered as a generic protein, our comparison suggests that the use of serum albumin as a model for structural proteins can be overestimated.
mass distribution model was used to estimate tissue-water distribution coefficients. Protein-water and lipid-water partition coefficients needed for this model were estimated using the PP-LER models. The results suggest that the protein fraction could have little contribution to the accumulation of hydrophobic compounds even in lean tissues. In contrast, a high contribution of the protein fraction is expected to the partitioning of some polar compounds to such lean tissues. The data and estimation models presented here will be useful to understand and estimate the accumulation capacity of organisms/tissues in varying compositions.

ET04-5
Interaction of environmental contaminants with P-glycoprotein from trout
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P-glycoprotein (P-gp) is a membrane protein acting as cellular efflux pump of structurally unrelated chemical compounds. It confers “multixenobiotic resistance” (MXR) of cells and has an important role as “active barrier”, preventing compounds from crossing tissue-compartment interfaces. There are two aspects of chemical interaction with P-gp: 1) By preventing chemicals from crossing compartment interfaces, such as from food into the blood-stream in the gut, bioconcentration rates are lower than would be expected from physico-chemical properties of a compound; 2) Chemical interaction with P-gp can lead to disruption of its function, thus rendering the P-gp barrier for chemicals dysfunctional. This could explain why bioconcentration of certain chemicals varies when present in combination with other compounds. There is recent evidence that P-gp from fish- in analogy to the well-studied homologs in mammals - also has properties as MXR transporter. However, test systems for functional studies of P-gp from ecotoxicologically relevant fish species are missing. We have recently established a cell line derived from RTL-W1, a rainbow trout (Oncorhynchus mykiss) liver cell line. The cells overexpress trout P-gp and were obtained by continuous exposure of RTL-W1 cells to low levels of vinblastine, a toxic P-gp substrate. Whereas P-gp expression in these cells, others, or in wild-type cells was regulated by the wild-type cell line. Thus, these cells provide the opportunity to study specific interactions of chemicals with trout P-gp. As expected, the selected cells are substantially more resistant to the toxic cancer drug, doxorubicine, than the wildtype cells, as determined in cytotoxicity tests after 3 days of exposure to different doxorubicine concentrations. The selected cells were also more resistant to the pesticides, rotenone and metachlor, indicating that P-gp activity may result in lower bioconcentration than would be expected. Pentachlorophenol and celestolide caused increased accumulation of the fluorescent transporter substrate calcine-am in the selected cells, indicating that those compounds disrupted P-gp activity, acting as chemosensitizers. Our selected trout cell line could be a useful component of a bio-tool kit for studies of ADME (adsorption-distribution-metabolization-excretion) processes of chemicals that are important to understand factors determining bioconcentration of chemicals in fish.

ET05 - Ecotoxicology and ecosystem services: a southern perspective
ET05-1
Regional scale risk assessment of threats to the yellowfish (Labeobarbus spp.) and the ecosystem services they provide in the in the Vaal River, South Africa's hardest working river
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The Vaal River is South Africa's most economically valuable aquatic ecosystems, and one of Africa's hardest working rivers. The ecosystem services provided by this system include a unique yellowfish dependent angling industry which is considered to be one of the largest and socially most valuable services of the system. Due to the excessive use of the Vaal River, many stressors occur which may pose a threat to the yellowfish populations of the Vaal River and the socio-economic values they offer to society. The nature and magnitude of these stressors affect the yellowfish populations of the Vaal River is largely unknown, and the current social and economic value of the yellowfish dependent angling industry in the Vaal River has not been documented. The aim of this study is to carry out a regional scale risk assessment of threats to the sustainability of the yellowfish in the Vaal River and associated social and economic value of the yellowfish dependent angling industry in the Vaal River. The approach adopted to reach the aims includes the application of the relative risk model to assess threats by multiple stressors to yellowfish populations in nine risk regions in the Vaal River. Thereafter a socio-economic assessment of the current and potential contribution of the yellowfish dependent angling industry in the Vaal River has been undertaken. This information has been used to assess the threats to the yellowfish in the Vaal River and the ecosystem services they provide. Findings show that yellowfish in the Vaal River are not only of ecological importance as a part of the biodiversity, but of great social and economic value to South Africans as well. The seasonal economic value of the yellowfish dependent angling industry was determined to be worth over US$16.7 million. Thereafter the study shows that due to the excessive use of the ecological services in the Vaal River, this value of yellowfish is at risk of being impacted on by chemical pollution, flow alterations, habitat alterations and disturbance to wildlife stressors. Although currently conditions may be acceptable, trends show that if increasing use of the ecological services of the Vaal River continue the likelihood that the biodiversity of our country would be threatened and that many South Africans would suffer social and economic losses. To avoid these losses and possibly enhance the value of yellowfish as an ecosystem service in the Vaal River existing environmental management regulations should be implemented.

ET05-2
Ecotoxicological study about the effects of Endosulfan in the viviparous fish Jenynsia multifilamenta (Anabilipeidae, Cyprinodontiformes)
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Environmental ecosystems such as purification of air and water are being impaired by many human activities such as unsustainable agricultural practices. The use of pesticides leads to the pollution of many aquatic habitats worldwide. Viviparous fish Jenynsia multifilamenta (Anabilipeidae, Cyprinodontiformes) is proposed as a bioindicator because its wide distribution along the Neotropical region of South America and inhabits polluted as unpolluted sites. The aim of this work was to develop an integrall analysis of pesticide effects and the interaction in J. multifilamenta. First, the acute toxicity of technical-grade Endosulfan sulfate (EDS) was determined and histomorphological alterations in gills and liver was described and quantified. Second, sublethal toxicity tests (0.014-1.4 μg.L-1) were carried out. As biomarkers of effect, response of detoxification and antioxidant systems (GST, GPx, OR, and CAT) and the effects on Lipid Peroxidation (LEO) were measured in different organs. Neurotoxic effects of EDS were evaluated through the AchE activity in brain and muscle. At behavioral level, swimming activity was recorded. As biomarkers of exposition, accumulation of technical-grade of EDS and Endosulfan sulfate (ES) was measured. The LC50-96 was lower in males (0.72 μg L-1) than females (1.12 μg L-1). The histomorphological alterations in gills such as epithelial lifting are considered as defense responses to minimize the entry of EDS into the blood stream. In liver, the histological alterations varied between irreversible changes at lower concentrations to reversible changes at the highest concentration. At biochemical level, liver and brain were the most damaged organs and presented the highest levels of LPO. At behavioral level, hypoxia was observed in relation to the exposure time. These swimming activity changes were associated with the inhibition of AchE activity in muscle modifying the normal behavior of fish. The accumulation of the metabolite ES in all tissues indicate biological transformation of EDS. Moreover, measurement of technical grade EDS as well as ES is suitable biomarkers of exposure under field conditions. If the stressful conditions persist, the mentioned alterations would lead to changes at population levels.

ET05-3
Effects of Chlorine onuger growth and food utilization in Australian catfish, tandanus tandanus
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2ETH Zürich, Zürich, Switzerland
The native Australian catfish tandanus tandanus, also called the “eel-tailed catfish is found in most river systems throughout the country and in pre-settlement times was a major food fish for indigenous Australians. It is an edible species that is still favoured by rural communities. It is also considered to have potential for aquaculture. However severe barriers to develop and aquaculture industry for this species have failed to date. The wild catch declined dramatically from “fairly abundant” during 1970-1971 to the species being declared “fully protected” in most of the regions of the country at present. Organophosphate (OP) pesticides are widely used in Australia and are therefore a common contaminant in the aquatic environment. These chemicals may have significant impacts on non-target aquatic species, including fish which are particularly sensitive to OP toxicants and though their effects on fish have been widely studied, in Australia, no study has been conducted on the effects of pesticides on the Australian catfish. Our study evaluated the effects of a pulse exposure of technical-grade chlorpyrifos followed by subsequent recovery in optimum conditions on the growth and feed utilization of Australian catfish, T. tandanus, simulating field pesticide exposure conditions. T. tandanus were exposed to a short term pulse of chlorpyrifos at 2 or 10 μg L-1 and grown in optimal conditions for 6 weeks. Growth and feed utilization of catfish were significantly impaired after exposure to chlorpyrifos. The hepato-somatic index of catfish exposed to 10 μg L-1 was significantly elevated compared with time post-exposure to chlorpyrifos in relation with the recovery of brain Acetylcholinesterase (AChE) activity. The results revealed that the fish were able to recover from the pulse of pesticide exposure since their growth rate was found to be as high as the control during the last 2 weeks. It is therefore possible, that poor growth performance resulting from exposure to OPs would be compensated for and fish recover if favorable conditions are prolonged. This implies the necessity for the reduction of repeated spraying of OP pesticides in the field and it is recommended that more such investigations be conducted on native, iconic food fish of cultural value.
High Hg concentrations were found in surface water (0.01 - 220.63 ng IHg L⁻¹ and 0.04 - 2.12 ng MHg L⁻¹) and sediments (46 - 2090 ng IHg g⁻¹ and 2 - 5 ng MHg g⁻¹) and reached concentrations of 0.01 - 220.63 ng IHg L⁻¹ and 0.04 - 2.12 ng MHg L⁻¹. This finding is significant as it demonstrates the higher concentrations of Hg are found at the study location.

ET05-5

Anthropogenic impacts on ecosystem services of a subtropical lake in southern Africa

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²North West University, Potchefstroom, South Africa

Pondlopaaplo (LP), situated in the sub-tropical northern KwaZulu-Natal, South Africa (SA), is the recreation angling hotspot of SA and one of the few places in SA to catch fish. To assess the impact of Hg pollution in the Wesr Rand areas with long history of gold mining and to investigate the Hg distribution, and characterize potential sources, pathways, receptors and sinks in order to implement mitigation strategies and minimize risk.

The purpose of this work was to carry out an assessment of Hg pollution in the Wesr Rand areas with long history of gold mining and to investigate the Hg distribution, and characterize potential sources, pathways, receptors and sinks in order to implement mitigation strategies and minimize risk.

Risk assessment of Hg pollution in SA is based on total concentrations which is not sufficient to understand its transport and fate. It is necessary to evaluate Hg speciation, as the lead has a higher impact on the health of LP tigerfish. The aim of this study was thus to quantify two of the most threatening anthropogenic impacts in South Africa.

The impact of gold mining on mercury pollution in the Witwatersrand Basin, South Africa

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Gold mining in the Witwatersrand basin, SA contains the biggest and richest mines in the world. Mercury, which occurs in some gold-bearing ores, was also used for gold recovery till 1915 and is still used in artisanal mining. Presently some old gold tailings dumps are reprocessed. Consequences of these activities are the release of Hg to the environment.

FT06 - Ecotoxicology of amphibiaean and reptiles. Novel approaches for linking contaminant effects with population declines

ET06A-1

Changes in morphology, behaviour and energy reserves of the African Toad Bufo regularis exposed to lethal and sublethal concentrations of Diazinon

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The lethal and sublethal toxicity of the insecticide diazinon on the African toad, Bufo regularis were evaluated to assess changes in morphology, behaviour and energy reserves. The median lethal concentration of the insecticide was found to be 0.44mg/L indicating the high toxicity of the insecticide. Uptake of the pesticide by the toads caused dose-dependent deformities and behavioural abnormalities. More pronounced poisoned symptoms were observed at higher concentrations. The pesticide caused differential increase in glucose levels in the blood with a concomitant reduction in liver glycogen. The increase in glucose levels and the reduction in glycogen levels indicate disorders in carbohydrate metabolism due to pesticide induced stress. The findings revealed that changes in morphology, behaviour, glucose and glycogen levels could be considered suitable biomarkers in evaluating the effect of diazinon.

ET06A-2

Combined effect of malathion and nitrate on survivability of tadpoles of Indian cricket frog fejervarya limnocharis

V. Krishnamurthy

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We have examined individual and combined effect of realistic concentration of Malathion and nitrate on the survivability of Indian Cricket Frog tadpoles. In this fully factorial experiment, tadpoles were subjected to individual and combinations of 0, 500, 1000 and 2000 μg malathion/Land 0, 2 and 4 mg NO₃-N/L. When tested alone, malathion found to be detrimental to tadpoles and reduces the survivability, while nitrate increase the survivability. Malathion was found to be lethal to tadpoles and reduces the survivability, while nitrate increase the survivability. The response exhibited at the combined treatment does not follow the trend recorded at different concentration of each of the tested chemical. Presence of nitrate enhances the effect of Malathion at lower concentration, while at higher concentrations of nitrate and Malathion, the trend of survivability was determined by Malathion alone. Since the breeding season and tadpole stage of this frog coincide with application of pesticide and nitrate, there is every possibility that these combinations could affect frog population in agro-ecosystems.

ET06A-3

Amphibians at risk ! Sustainability of terrestrial amphibian life stages to pesticides

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¹Institute for Environmental Sciences, Landau, Germany

We have examined individual and combined effect of realistic concentration of Malathion and nitrate on the survivability of Indian Cricket Frog tadpoles. In this fully factorial experiment, tadpoles were subjected to individual and combinations of 0, 500, 1000 and 2000 μg malathion/L and 0, 2 and 4 mg NO₃-N/L. When tested alone, malathion was found to be detrimental to tadpoles and reduces the survivability, while nitrate increase the survivability. The response exhibited at the combined treatment does not follow the trend recorded at different concentration of each of the tested chemical. Presence of nitrate enhances the effect of Malathion at lower concentration, while at higher concentrations of nitrate and Malathion, the trend of survivability was determined by Malathion alone. Since the breeding season and tadpole stage of this frog coincide with application of pesticide and nitrate, there is every possibility that these combinations could affect frog population in agro-ecosystems.
Effects of a mixture of agricultural pesticides and estrogenic compounds on developing tadpoles

ET06A-4

Ranking ecological risk of multiple stressors on amphibians

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In this study we rank the potential threats to amphibians in their actual and potential habitats in the Netherlands. The study combines laboratory toxic effect data (acute median Lethal Concentrations, LC50) for anuran species with environmental monitoring data for different stressors and actual survey data of anurans in the Dutch freshwater bodies. Combining all the data together, the ecological risk (ER) was calculated for ammonium, nitrate, cadmium, copper, pesticides (18 different compounds), and acidity. For each stressor we predicted a probability that anuran species are exposed in the field to stressor concentrations exceeding their LC50. The total ER at the LC50 level, as calculated for Dutch freshwater sites was found to be 2.73%. We ranked the stressors in decreasing order of their ER: pH, copper, diazinon, ammonium, and endosulfan. Taking into account the bioavailability of copper, the ER for it might be reduced. Therefore, the main threats affecting anuran populations arise from acidification of their habitats, ammonium, and certain pesticides exposures. These results are valuable for conservation management of amphibians when prioritization for mitigation of stressors is needed. The applicability of deriving the ER is a useful tool for location-specific risk assessment of multiple stressors for selected amphibian species and can be applied in other locations for species of concern.

ET06A-5

Are standard avian risk assessments appropriate tools addressing the risk to reptiles?

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According to the new data requirements under the current EU regulation 1107/2009, ′available and relevant data, including data from the open literature for the active substance of concern, regarding the potential effects of an active substance to birds, mammals, reptiles and amphibians should be presented and taken in to account in the risk assessment.′

Whereas for birds, mammals and amphibians specific data requirements are available, nothing is stipulated for reptiles. As stated in the Guidance of EFSAs − Risk Assessment for Substances of Environmental Significance (2010): ′Information should be provided for the substances of concern, regarding the potential effects of an active substance to birds, mammals, reptiles and amphibians should be presented and taken in to account in the risk assessment.′

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ET06B-1

Thermal tolerance of amphibians and their invasive predator in a polluted environment

T.C. E. Kullgren, E. Karakoti, N. Lutzmann, R. Lehmkuhl

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It is clear that many threats such as habitat loss, chemical pollution and global warming are contributing to both local and global amphibian declines. Yet aside from a few examples, we still know very little about the role and magnitude of synergistic effects among these stressors. Demonstrated in marine ecotones, thermal tolerance is limited by the incapability of an organism's circulatory and ventilation systems to supply sufficient oxygen to match with its oxygen demand (i.e. aerobic scope). Beyond certain critical temperatures, survival of aquatic ectotherms becomes passive and time-limited, relying heavily on anaerobic metabolism and molecular protection (e.g., induction of heat shock proteins). Detoxification is known to be an energy-demanding process. It is thus likely that an organism under thermal stress will suffer further decrease in aerobic scope and loss of performance in a contaminated environment, which may in turn threaten their survival. Our study thus focused on the relationships between pollution and thermal stress on three lowland wetland amphibian larvae in South China, namely Asian common toad (Bufo bufo), and the invasive predatory grass snake, Natrix natrix.

ET06B-2

Effects of a mixture of agricultural pesticides and estrogenic compounds on developing tadpoles

4 Harlan Laboratories Ltd., Inningen, Switzerland
5 Federal Environment Agency, Dessau, Germany
6 ZALF eV, Muencheberg, Germany

The global amphibian decline1 is manifested with more than 30% of all amphibian species on some level of threat according to the IUCN. Pesticides are considered one factor for the reduction of species and observed population declines. Pesticides reach aquatic habitats by drift and runoff whereas they are applied at high field rates in crops where they adhere to soil particles and plants. Although amphibians are depending on water bodies for their development the majority of their adult life cycle is spent in the terrestrial habitat. So far no specific risk assessment is carried out for the terrestrial life stage of amphibians since it is assumed that they are covered by the procedures in place for birds and mammals.

We present data for presence of amphibians in two different agricultural landscapes in Germany and discuss the difference in skin properties and uptake between amphibians and mammals. An extensive review (Brühl et al. 2011) revealed that only few toxicological data exist for amphibians that were conducted to mimic an overspray scenario and dermal exposure of amphibians. This research gap was filled by conducting rate-response test with different pesticides and juvenile Common Frogs (Rana temporaria) using a spraying chamber and realistic field application scenarios. Observed mortalities at rate-response fields were situated between 20 and 100%. There was no difference between fungicides, insecticides and herbicides. Furthermore it remains unclear if the observed toxicity is based on the active substances or the solvents in the formulations or a combination of both.

We conclude that terrestrial life stages of amphibians are present in agricultural landscapes and crops during application of pesticides. Passage of pesticides through a per capita lethal temperature is as well as for mammals. The investigation on the synergistic effects of thermal stress and pesticides is currently being conducted using integrated measurements of physiological end-points and biomarkers including growth rate, oxygen consumption rate, physiological end-points and biomarkers including growth rate, oxygen consumption rate, and predator (herbivorous, omnivorous, insectivorous and carnivorous). None of the detected amphibian and reptile species is exclusively herbivorous. All of the detected amphibians and the most lizards can be assigned to the insectivorous guild. Only the Mediterranean turtle (Mauremys leprosa) and the ocellated lizard, Timon lepidus, have to be treated as omnivorous. All of the snake species feed on vertebrates. Available habitat data given in the IUCN Red List (IUCN 2011) and by Fryday & Thompson (2009) was compared to our findings per species. The grass snake, Natrix natrix, was the only species that might be at risk of exposure by PPPs according to Fryday & Thompson (2009), and found during our surveys. Because of the obvious differences between published data and our observations further fieldstudies are needed to obtain quantitative data on species compositions of the herpetofauna and densities of possible focal species for risk assessments for IPPP in agricultural land in Spain.

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Acute and chronic toxicity tests with the Australian striped marsh frog (Limnodynastes peronii) and the cane toad (Rhinella marina) were undertaken to determine if the combination of common agricultural pesticides (glyphosate, atrazine, endosulfan) and estrogenic compounds (4-nonylphenol and estradiol) at environmentally realistic concentrations affect tadpole development and survival. There is a growing concern among scientists and the agricultural and chemical industries about the potential effects of agrochemicals on amphibian populations. An important consideration in the study of populations is the assessment of the interactions of the environment with the genetic susceptibility of the individuals'

**ET06B-3**

Effect of the acute exposure (96 h) to xenoestrogens on *Lithobates catesbeianus* tadpoles


Federal University of São Carlos, Sorocaba, Brazil

The aim of this study was to evaluate if a combination of 17α-ethynylestradiol (EE2) normally found in Brazilian industrial effluents can exert some impact on the cardiac function of bullfrog tadpoles (25 Gosner stage), *Lithobates catesbeianus*. To this end, animals were exposed for 96 h to 10 nM of EE2 and their responses were compared to controls. During exposure, the animals' activity level (AL % of active individuals) was monitored twice a day. Immediately after exposure, the in loco heart rate (fH - bpm) was determined. Afterwards, animals were sacrificed and the ventricles were removed for the preparation of ventricular strips to record isometric twitch and contraction amplitudes. The results indicated that EE2 did not affect tadpoles' AL (% of active individuals, n = 52 bpm). These findings suggest that EE2 did not affect cardiac muscle of bullfrog tadpoles, rather than its effects being a result of an increased cardiac response due to a higher activity level in order to the aversive stimulus of the exposure to the xenoestrogen (i.e., avoidance response). Additionally, the comparison between the values of AL between the two experimental groups showed that the xenoestrogen exerted a positive inotropic response. Thus, it can be suggested that the increase in cardiac performance induced by the exposure of bullfrog tadpoles to this xenoestrogen elevates considerably the animal energy expenditure, diverging a large amount of energy towards tadpole growth for their growth and development. These alterations make the animals more susceptible to predators and also reduce the likelihood that they will reach reproductive stage.

**ET06B-4**

Novel methods to assess health of amphibian populations: preliminary results

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New methods to assess the health of amphibian populations have been developed, such as those that use biomarkers of exposure (induction of CYP1A in skin biopsies, accumulation and excretion of porphyrin) to biomarkers of indirect and direct effects by investigating responses. The analysis of results by classes of age showed that levels of PAHs and Hg tend to increase with age. The same trend was found for lipid peroxidation and ENA assay results. As well as high comet assay response and total PAH levels were found in two young specimens sampled off-shore of the Calabrian coasts (Brancaleone) after an oil spill. The presence of estradiol appeared to have little influence on these endpoints. Endosulfan was found to be the most toxic compound in our mixtures, causing erratic swimming behaviour and darkening of the skin following exposure to this compound alone and in mixture. However, the acute toxicity was only enhanced with the presence of sub-lethal concentrations of the other compounds. Further research will help identify specific mixtures and chemical ratios causing effects, but our results support the hypothesis that complex mixtures at sub-lethal concentrations cause detrimental effects on developing tadpoles.

**ET06B-5**

Screening breeding ponds of the Common toad (Bufo bufo) in the UK for evidence of endocrine disrupting activity

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To date, there has been no attempt to evaluate the potential impacts of endocrine disrupting contaminants of the aquatic environment on wild populations of native amphibians in the British Isles, though this group has suffered declines in the agricultural landscape. We selected nine breeding sites of the Common toad (Bufo bufo) in the UK on the basis of predicted surface concentrations of agrochemicals and other potential contaminant hotspots based on counts of adult toads in the breeding migration. Samples of spawn were endocrined, as well as individuals with liver damage, were monitored though larval development. Larvae and toadlets were sampled at several times point for gonadal histopathology at five of the study sites. We also deployed semi-permeable membrane devices (SPMD) and Polar Organic Chemical Integrated Samplers (POCIS) at all sites for 4 weeks and screened extracts for endocrine disrupting activity in recombinant Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS), and in primary monolayer cultures of *Xenopus* hepatocyte monolayer cultures of *Xenopus* at all sites for 4 weeks and screened extracts for endocrine disrupting activity in recombinant Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS), and in primary monolayer cultures of *Xenopus* monolayer cultures of *Xenopus* for gonadal histopathology at five of the study sites. We also deployed semi-permeable membrane devices (SPMD) and Polar Organic Chemical Integrated Samplers (POCIS) at all sites for 4 weeks and screened extracts for endocrine disrupting activity in recombinant Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS), and in primary monolayer cultures of *Xenopus* monolayer cultures of *Xenopus* for gonadal histopathology at five of the study sites. We also deployed semi-permeable membrane devices (SPMD) and Polar Organic Chemical Integrated Samplers (POCIS) at all sites for 4 weeks and screened extracts for endocrine disrupting activity in recombinant Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS), and in primary monolayer cultures of *Xenopus* monolayer cultures of *Xenopus* for gonadal histopathology at five of the study sites. We also deployed semi-permeable membrane devices (SPMD) and Polar Organic Chemical Integrated Samplers (POCIS) at all sites for 4 weeks and screened extracts for endocrine disrupting activity in recombinant Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS), and in primary monolayer cultures of *Xenopus* monolayer cultures of *Xenopus* for gonadal histopathology at five of the study sites. We also deployed semi-permeable membrane devices (SPMD) and Polar Organic Chemical Integrated Samplers (POCIS) at all sites for 4 weeks and screened extracts for endocrine disrupting activity in recombinant Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS), and in primary monolayer cultures of *Xenopus* monolayer cultures of *Xenopus*.
Environmental proteomics to predict the effects of global warming on aquatic organisms exposed to pollutants

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ET07A-1

Proteomics studies in ecotoxicology have the potential for the detection and quantification of proteins related to chemical stress. These proteins might serve as novel biomarkers for a rapid expansion into toxicological proteomics. As studies based on 2-DE are comparatively elaborate, time-consuming and expensive, the finding of robust biomarkers is limited by the numbers of replicates, statistical methods and also by the experimental design. For these reasons, many published proteomics studies in the area of (eco-) toxicology are confined to one or two tested concentrations. As reactions at the molecular level are expected to be a function of exposure concentration, the analysis of low concentrations might direct the experiment to fragmentary or incomplete conclusions. In the present study an experimental design for a proteomics study was tested for detection of induced protein regulation. This design included the analysis of concentration dependence without increasing the replicate number of 12 parallel 2D-gels. The tests were done using the zebrafish embryo as a vertebrate model of teratogenicity. To test whether the proteome analysis is able to discriminate between substances with different mode of action (MoA), the substances Phenanthrene (narcotic MoA) and Gemfibrozil (specific MoA) were selected for the proteomic tests. For the 2D-GE electrophoresis experiments, Danio rerio embryos were exposed to six different concentrations ranging from 1% of LC50 to the LC20 (Gundel et al. 2011). The selected concentrations were based upon a teratogenicity assay with zebrafish embryos accomplished in closed glass vessels. Proteomics analyses were done by 2D-DIGE technique (Marouga et al. 2005) with minimal CyDye labeling. Protein abundance profiles of around 700 signals were studied in the Phenanthrene exposure tests. About one third of the protein spots could be detected to show reactions correlating with stressor concentration. From this group about 70 protein spots already showed changes beginning at low and very low effect concentrations. In this study it could be shown that increasing the number of measured concentrations instead of replicate number of 2D-GE electrophoresis experiments, the detection of robust stress markers was possible. From these proteins many may be already detectable at very low lethal effect concentrations such as the LC01. In this way the possible output of proteomics experiments may be increased considerably.

ET07A-2

The Concentration-Response Concept in Proteomics: effects of a narcotic (Phenanthrene) and a specific acting Substance (Gemfibrozil) to the Proteome of Zebrafish Embryos (Danio rerio)

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ET07A-3

Integrated environmental genomics of cod (Gadus morhua) reveal the mechanisms underlying MeHg- and PCB 153 induced toxicity

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Atlantic cod (Gadus morhua) is an important species in both North-Atlantic fisheries and aquaculture. However, coastal cod populations are highly stationary, and are therefore particularly susceptible to environmental insults. Coastal- and petroleum industry are expanding into cod habitats, and limited information exist on the effects on cod to both acute- and long-term exposure to several environmental contaminants. Importantly, in order to monitor and maintain sustainable coastal cod population, it is necessary to understand how such contaminants may affect growth, reproduction, and health of this species. Methylmercury (MeHg) and PCB 153 have several properties in common. Both compounds act as neurotoxins, they are persistent environmental pollutants, and ubiquitous contaminants that are biomagnified in aquatic food chains. To develop a deeper understanding of transcriptional and translational responses of the cod genome to MeHg and PCB 153, we have initiated a toxicogenomic approach combining transcriptomics, proteomics, and bioinformatics, as an attempt to integrate these responses into mechanistic insights. The liver proteomes of MeHg and PCB 153 exposed cod have been resolved with both gel-based and LC-MS/MS based methodologies, while the corresponding liver transcriptome from these samples have been analyzed with microarray analysis using a cod-specific array. We have simultaneously established an in-vitro exposure system using cod liver slices for accompanying the in-vivo exposure experiments. Moreover, this interdisciplinary study has provided detailed information regarding the modes of action of both MeHg and PCB 153, including responses to less severe conditions previously not known. The differentially regulated proteins are also candidates for new and more sensitive biomarkers for MeHg and PCB 153-exposure for use in environmental monitoring and risk assessment. The project iCod: integrative environmental genomics of cod (Gadus morhua) is funded by the Norwegian Research Council (project 192441/I30). Thanks to the Genofisk Consortium and the Cod Genome Sequencing Project Team for sharing data in advance of the public release of the cod genome data (www.codgenome.no).

ET07A-4

In vitro exposure of nanoparticles in mouse: an integrated proteomic and lipidomic study

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ET07A-5

Altered protein expression in stickleback (Gasterosteus aculeatus) gills after silver nanoparticle exposure

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Beyond shifts in species distribution, most organisms are forced to acclimate or adapt to long-term changes in temperature and frequently exhibit temperature compensation mechanisms. Moreover, aquatic species are also subjected to polluted environments. It is consequently necessary to assess the combined effects of heat stress and xenobiotic exposure for predicting the impacts of global warming on wildlife. We report here the results from two studies on the proteomic responses in fish species under combined heat stress and pollutant exposure. The first study aimed at identifying proteins whose expression has been modified by fire temperature and selenium (Se) exposure in larval green (GS) and white sturgeons (WS). The aim of the second study was to investigate whether acclimation to 3 temperatures may lower or enhance the ability of European bullhead to tolerate subsequent exposure to cadmium (Cd). In GS and WS, gel replicates were first grouped according to heat treatment. Proteins involved in protein folding, protein synthesis, protein degradation, ATP supply and structural proteins changed in abundance in response to heat and/or Se. In bullhead, thermal acclimation was the first parameter affecting the protein expression profile, while fish’s ability to respond to Cd was clearly affected during acclimation to 21°C. The identified differentially expressed proteins were associated for instance with protein turnover, folding and chaperoning, transmembrane transport, metabolic regulation, cell signalling, and cytoskeletal reorganization. This work provides insights into the interactive effects between heat acclimation and a subsequent exposure to Cd, and suggests that further studies on the identified proteins could offer essential information to better understand the mechanisms of action shared by these two environmental stresses. In conclusion, we showed using the model that proteomics can be a powerful tool to investigate the effects of heat stress on the cellular phenotype. A specific proteome could be defined for each stress condition, and likely represents the cellular responses to specific mode of action. When cross-tolerance is observed, those proteomic signatures can bring clues to understand shared mode of action. The opportunity to focus on this cellular phenotype acquired during acclimation is discussed in the perspective of evolution theories and should be taken into account in order to predict the effects of global warming on wildlife.
Engineered nanoparticles (ENPs) are rapidly becoming an important part of new technology and are today used in a variety of products, from computers to pharmaceuticals and body care products. Silver nanoparticles (AgNP) are the most widely used nanoparticles, especially due to its antimicrobial properties. AgNP may be introduced into the environment via consumer products containing silver nanoparticles, for example in personal care products. The oral ingestion of AgNP that needs to be elucidated including its toxicological function. Silver ions are toxic to fish due to their ability to bind to thiols groups inhibiting enzymes such as Na+/K+ ATPases that are extremely important to the gut osmotic function. In order to study the effects of AgNP in fish, sticklebacks (Gasterosteus aculeatus) were exposed to AgNP (0.1 mg/L) and silver ions (0.01 mg/L) in a freshwater semi-static system for seven days. Gill samples were taken for proteomic studies, transmission electron microscopy (TEM) and gene expression analysis on a 1000K microarray. The behavioral (e.g. agglomeration rate) of the AgNP in the test water and levels of sonic and total silver were monitored throughout the experiment. Amine-reactive TMT Isobaric Mass Tagging was used to quantitatively label proteins extracted from gills for identification and analysis by mass spectrometry (TMT sixplexTM label reagent set, Thermo Scientific). Proteins were identified with the help of a Stickelback specific proteome database. Ionic silver exposure resulted in more regulated protein expressions compared to the AgNP exposure. 116 proteins were regulated in the Ag+ exposed gills (33 down and 62 up) while only 43 were regulated in the AgNP exposed gills (22 down and 21 up). Among the regulated proteins only nine proteins were equally affected by both treatments meaning that 34 of the observed protein regulations were AgNP specific. Several of the regulated proteins in the AgNP exposed fish have a function in cell structure and cytokinetics. Also, a mucus forming glycoprotein (mucin 2) was up-regulated in the AgNP exposed fish suggesting that the gill epithelial cells were stressed. The presence of AgNP in gill cells was detected using TEM. This study was supported by FORMAS and proteomic analysis performed in collaboration with the proteomics core facility at Gothenburg University.

ETD7A-6

Transcriptomic responses in Japanese medaka (Oryzias latipes) exposed to individuals or mixtures of polycyclic aromatic hydrocarbons: mixture toxicities or fasting effects?

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In the present study, we assessed the effects of single or mixtures of polycyclic aromatic hydrocarbons (PAHs) by transcriptome analysis. Four-week-old Japanese medaka (Oryzias latipes) were exposed to phenanthrene (5.50 ppb), pyrene (3.30 ppb), or their mixtures for 96 hours. Fish were sampled at 0, 24, or 96 hours after the exposure test started. Total RNA was extracted from whole body of medaka was subjected to microarray experiment. Primary component analysis of transcriptome data at 24-hr exposure showed that three groups were formed and were separated from each other, indicating that PC1 (32.1%) and PC2 (12.5%) reflected the effects of mixtures and single PAHs respectively. The profiles of altered gene expression at 24-hr exposure were similar between phenanthrene- or pyrene-exposed groups; however, the effects of their mixture were completely different from those of individual compounds. Exposures to phenanthrene or pyrene induced the genes related to immune response (GO:0006955) and induction of apoptosis (GO:0006917) in a dose-dependent manner, which seemed to be an acute response to PAH exposures. Meanwhile, exposures to mixtures of phenanthrene and pyrene dramatically suppressed the expression levels of genes associated with collagen catabolic process and glycoysis (GO:0006969), although the PC1 result did not show the similarity trend. On the other hand, the PCA result of 96-hr exposed groups did not show the similar trend, and the gene expression patterns exhibited neither dose-response nor PAH mixture-specific effects. The effects of fasting, detected as significant changes in gene expression profiles of control fish among time points. Data analyses using GO terms revealed that 96-hr fasting significantly suppressed collagen catabolic process and glucose homeostasis (GO:0042593), which were also observed in fish exposed to mixtures of PAHs. The effects of fasting seemed larger than those of PAH exposures at 96-hr, which may affect the mixture response with the single PAH exposures. Thus, our analysis can detect mixture toxicities of PAHs, as well as the effects of individual PAHs. However, some effects of PAHs were overlapping with the effects of fasting. Therefore, for precise evaluation of chemical effects by microarray experiment, fasting should be considered as a factor to affect the gene expression profiles in experimental animals.

ETD7B-1

Transcriptomics analysis of intersex condition in rainbow darter (Etheostoma caeruleum) exposed to sewage effluents in Grand River, Ontario, Canada

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Rainbow darter (Etheostoma caeruleum) is a small benthic fish found in North America. Rainbow darters are abundant and spatially distributed throughout the Grand River basin in Ontario, Canada. The present study is focused on the effects of exposure to sewage effluents on the expression patterns of genes associated with the development of intersex condition. The aim of the present study was to develop a microarray expression microarray and compare the sensitivity of gene expression endpoints to life-cycle endpoints in sediment dwelling C. riparius larvae. Therefore, we performed 14-day sediment toxicity tests with four compounds, i.e. the essential metal copper, the non-essential metal cadmium, the organometal tributyltin and the polycyclic aromatic compound phenanthrene, and measured the effects on growth and survival. EC50 and LC50 values were obtained for all compounds, except for phenanthrene where the surviving larvae were not impaired in their growth, thus no EC50 value could be calculated. To obtain the C. riparius transcriptome, a portion of the genome size (70%) was sequenced on an Illumina HiSeq2000 sequencer yielding 1549146 sequence reads which were assembled into 23709 isotigs and 135082 singletons. BlastX search and functional annotation with Blast2GO showed that 83% of the isotigs had the highest homology with dipterans, while 92% matched best with insects in general. In order to study the cDNA library of E. caeruleum (Grand River) we isolated and sequenced cDNAs from cDNA libraries from different time points. The microarray analysis between males from reference sites, males from polluted sites and intersex males demonstrate that the intersex condition in teleosts.

ETD7B-2

Sequencing the Chironomus riparius transcriptome to compare the sensitivity of gene expression and life-cycle endpoints to toxic exposure

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The Chironomus riparius (Diptera, Tipulidae) is a model system for the study the sensitivity of effets on fish populations because of high municipal sewage input. Rainbow darters are sensitive to sewage effluents in this environment, showing the presence of intersex condition in males. The primary objective of this research was to identify molecular pathways that are associated with intersex and to explore the effects of sewage effluent on higher level biological endpoints. In 2011, we collected female and male rainbow darters at 9 sites in Grand River in a gradient of sewage effluent pollution. There were no significant changes in condition factor, hepatosomatic index (LSI), or gonadosomatic index (GSI). Fish in polluted sites had lower body condition than the fish in reference sites. There was a high incidence of intersex in polluted sites as characterized by the presence of follicles within the testes. Male fish had less 11-Keto-testosterone and testosterone production at the downstream sites. To better understand understanding intersex in the rainbow darter, known genes involved in sexual differentiation (sox9b, fox1d2 and dmrt1) and reproduction (esr1, erib, vsf, aromatase and cypl1a) were cloned and evaluated with real-time PCR, comparing males, females and intersex fish. In addition, a rainbow darter microarray was developed using 454 pyrosequencing to characterize molecular pathways that are involved in intersex. For example, Sox9b and foxl2 were significantly up-regulated in the intersex condition while dmrt1 keep express on intersex. Vtg expression was significantly increase in intersex in comparison with males from reference and polluted sites. Cluster analysis of microarray data shows individuals that have a more advance intersex condition (i.e. vitellogeninc oocytes) have a very unique gene expression pattern compared to males and females. The microarray analysis between males from reference sites, males from polluted sites and intersex males demonstrate that the biochemical processes that included oogenesis were different in intersex compared to males. Finally, this study identifies novel genes and cell pathways underlying intersex condition in teleosts.

ETD7B-3

A novel method for cross species gene expression analysis - applications for ecotoxicology

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Cross-species comparisons and extrapolations is a cornerstone of ecotoxicological risk assessment. A general method that could be applied to the growing number of ecotoxicologically relevant transcriptomic studies would therefore be highly valuable in the identification of evolutionary conserved modes of action as well as novel biocandidate effects. Indeed, cross-species comparison is a powerful way to identify evolutionary conserved transcriptional responses. However, due to evolutionary events such as gene duplication, there is no one-to-one correspondence between genes in different species and comparison of their gene expression profiles is therefore complex. Here, we have developed a new method for cross-species meta-analysis of transcriptomic data. In contrast to other procedures, our method takes the homology structure between compared species into account and can therefore compare expression data from genes with any number of orthologs and paralogs. A simulation study shows that the proposed method results in a substantial increase in power compared to other previously suggested procedures. As a proof of concept, we analyzed microarray data from heat shock experiments performed in eight species where we identified several well-known evolutionary conserved transcriptional
responses. The method was also applied to five different gene expression studies where fish had been exposed to estrogenic substances. Well-known biomarkers of estrogenic exposure and mechanisms of action were identified together with more novel findings.

ET07B-4 Metabolomic approach to evaluate the effects of polybrominated diphenyl ethers (PBDEs) on the central nervous system and the hypothalamic-pituitary-gonadal axis of marine medaka (Oryzias melastigma)

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Owing to the extensive applications of polybrominated diphenyl ethers (PBDEs) as flame-retardants, residues of PBDEs can be easily found in the aquatic environment and biota. Resulting from their lipophlic characteristics, PBDEs can bio-accumulated bio-magnified along the food chain. Although great efforts have been devoted to the study of the environmental and toxicological impacts of PBDEs on the coastal marine ecosystem, our knowledge on their general health effects on marine biota still remains obscure. Because the central nervous system (CNS) is a primary target of these classes of compounds, the effects of PBDEs need to be investigated. Furthermore, Because of the intertwined nonlinear and dynamic interactions among large numbers of cellular components, such as genes, proteins and metabolites, organisms often respond to external stresses and stimuli in complex and unpredictable ways (Nicholson & Lindon, 2008). In order to understand the systemic responses and behaviors of a biological entity, an integrative approach to study and model the pathways and networks involved in the overall functioning of the entity is needed (Henry et al., 2003). Such a systems biology approach usually involves the perturbation of the biological system and monitors the resulting impacts at the various ‘omics’ levels, such as its genomics, proteomics and metabolomics.

In this study, we used marine medaka (Oryzias melastigma) as a marine vertebrate model organism to study the changes induced by the exposure to BDE-47, a predominant PBDE residue in the marine ecosystem, on the profiles of selected neuro-transmitters and their metabolites. By using a novel LC-ESI-MS/MS metabolomics platform, we were able to evaluate the multivariate metabolomic response on central nervous system (CNS) and hypothalamic-pituitary-gonadal (HPG) axis of a living system to PBDEs exposure. Our results indicate that food-borne exposure to BDE-47 is able to induce abnormal expression of a number of neuro-transmitters in the central nervous system of the model organism.

ET07B-5 Metabolomic approach to study the mechanisms of toxicity of a non-ionic surfactant in fish

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Non-ionic surfactants account for 50% of the total surfactant production and they are the second highest synthetic surfactant in terms of worldwide consumption. Between them alcohol polyethoxylates (AEOs) have the highest production volume in EU today, and they have replaced alkylphenol polyethoxylates (APEOs) due to the toxicogenic properties of some of their metabolites. After their use, AEOs are released either directly or via wastewater treatment plants into aquatic ecosystems assuming minimal risk for the health of aquatic life. Although AEOs do not present estrogenic properties as APEOs, there is little information on the effect of exposure to sub-lethal concentrations and the possible disruption on biochemical pathways of organisms that could adversely affect their health. In order to investigate this topic, a metabolomic profiling approach was used to characterise changes in tissues and biofluid of Solea senegalensis exposed to the non-ionic surfactant hexaethylene glycol monododecylether (C12EO6). Fish were exposed to two different concentrations of the chemical in a flow-through system for 120h, followed by a 72h of depuration period. Blood, liver and gills were extracted with methanol and the metabolites present in the extracts were profiles using LC-TOF-MS. Analysis of extracts revealed a high number of metabolites produced from the phase I and phase II biotransformation of the parent compound by the organism. Metabolomics analyses also revealed that the biochemistry of internal molecules was affected by the surfactant exposure. Increased concentrations of cortolone-3-glucuronide, and bile acids were measured in plasma and identified as potential biomarkers of surfactant exposure in marine medaka. The damage caused to the organism was still present after depuration of 72 hours indicating effects on the metabolism of fatty acids. After the depuration the levels of L-palmitoylcarnitine decreased by up to 10-3 fold as a result of surfactant exposure indicating affects on the metabolism of fatty acids. After the depuration the levels of L-palmitoylcarnitine were re-established indicating that the normal metabolism of fatty acids was recovered but the concentrations of bile acids kept increasing which indicate that the damage caused to the organism was still present.

ET07B-6 Metabolomic of and adaptation to arsenic in the carthworm, Lumbriicus rubellus
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As contamination in the terrestrial environment can have deleterious effects upon the flora and fauna exposed. The persistent nature of the metalloid provides a strong selective pressure that may lead to the occurrence of adapted populations. While certain organisms demonstrate tolerance, others are able to acquire genetically based resistance, whereby survival is ensured following exposure to concentrations that are normally lethal. Such resistance may come at a price, with reduction in genetic variability following selection events potentially increasing the sensitivity of adapted species to future environmental stress, while diverting resources limiting overall fitness. It is therefore important to determine the sensitivity of populations and potential ecological implications of resistance. Our investigations compare exposure sensitivity to metals among naive and tolerant populations of the carthworm Lumbriicus rubellus and evaluate the capacity for gene flow within contaminated environments via integration with phylogenetic data. Furthermore, we are employing a systems biology analysis (metabolomics and transcriptomics) to clearly resolve As metabolism within naive L. rubellus that will supplement investigations for the demonstration of mechanistic mechanisms adopted by adapted individuals using Restricted Associated DNA sequencing (RADseq).

ET08 - Extrapolation within wildlife toxicology

ET08-1 Increasing the reliability of the chemical effect assessment for wildlife with interspecies correlation estimations
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The median hazardous dose of a chemical for warm-blooded species (HD50) is one of the key characteristics in the relative ranking of toxic chemicals. Generally, extrapolation information is available for a limited number of warm-blooded species only, which causes statistical uncertainty. Furthermore, when small datasets contain an unrepresentative sample of species, they can cause systematic uncertainty in chemicals' median lethal doses. The number of species can be enlarged with interspecies correlation estimation (ICE) models, but these are uncertain themselves. The goal of this study is to quantify the possible gain in reliability of the HD50 values for warm-blooded wildlife species after enlargement of the sample size with ICE predictions. For 1137 chemicals, we compared potential systematic uncertainty and statistical uncertainty between HD50s based on experimental data (HD50Exp) and datasets combining experimental data and ICE predictions (HD50ICE). HD50ICE values ranged between 0.10 and 9,500 mg/kg-w-1, and HD50Co between 1.1 and 6,100 mg/kg-w-1. For over 97 percent of the chemicals, HD50ICE values exceeded HD50Co values, with a systematic uncertainty (i.e. the ratio of HD50Exp/HD50Co) of typically 3.5. This finding suggests that the limited availability of mammalian, experimental toxicity data results in a systematic underestimation of the chemical toxicity of a chemical. Statistical uncertainty factors (i.e. the ratio of the 95th/5th percentile) ranged between 4.8-100 and 1.0-2 for the experimental dataset, but between 1.0-100 and 2.5-1022 for the experimental dataset. For thirty percent of the chemicals, enhancement of the toxicity with ICE predictions gave a reduction in statistical uncertainty of up to twenty orders of magnitude, for the remaining seventy percent a combined dataset resulted in statistical uncertainty of maximally two orders of magnitude. The supplementation of experimental toxicity data with ICE predictions makes it possible to eliminate large outliers of statistical uncertainty. We conclude that both systematic uncertainty in chemicals' HD50 values and statistical uncertainty can be reduced by supplementation of experimental data with ICE model predictions, particularly in cases of limited toxicity data (n ≤ 4) for mammals only.

ET08-2 Contribution of wintering area to levels of organochlorines and polybrominated diphenyls in plasma of an avian top predator in the North Atlantic
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Top marine predators, including seabirds, are often sampled to monitor levels of persistent organic pollutants (POPs) in the marine environment. Despite the restrictions on use and production of many POPs and declines in some legacy POPs in the environment, concern remains about levels of these chemicals present in marine biota due to their potential toxicity. Many species of seabird are migratory, but there is a lack of studies investigating the possible contribution of time spent in their wintering area to their POP burden. Advances in tracking technology now mean it is possible to link the POP levels in an individual bird to their wintering area, recorded using geolocators. The great skua (Stercorarius skua) was chosen as the study species as it is a top predator and has breeding populations that contain individuals which winter in geographically discrete locations over a large geographic area. Geolocators were deployed in three breeding colonies in 2008, in Scotland (Foula; n=16), in Southeast Iceland (Oræfi; n=40) and in
ET03-3

Responses of wild small mammals to arsenic polluted soils on a partially remediating mining site in Southern France

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Arsenic (As) is a chemical element naturally occurring in soils but industrial and mining activities have long been releasing high As levels into the environment. The highest concentrations might affect directly exposed organisms and also organisms that feed on them along the food chain. Despite this, little is known about As behaviour in the environment. Chemical analyses of soils provide information on contaminant concentration but not on bioavailability, transfer, and toxicity to wildlife, all being critical points for ecological risk assessment (ERA). In As exposures, transfer of As between soils and organisms occurs at different scales. In contaminated soils, different pathways might be involved in the bioavailability and transfer of As to other organisms. Therefore, the study of the spatial relationships between soils and organisms might be a reflect of the foraging activity of animals. The analysis of small mammal diet, currently in progress, should help to test this hypothesis. Finally, the high internal As concentration call for an analysis of the effects of As on small mammals, both at the individual and population levels, which are also in progress and will be soon available.

ET04-8

Assessing the exposure and effects of persistent contaminants in river otters (Lutra canadenis) in Victoria Harbour, Vancouver Island, British Columbia

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4Monitoring contamination in wild otter populations has proven challenging due to their elusive nature and the invasive sampling techniques traditionally involved (e.g. trapping and tissue sampling). Elliott et al. (2008) recently reported that scats collected from river otter latrines in Victoria Harbour, BC, Canada contained levels of PCBs that exceeded 9 mg/kg lipid, a published criteria for reproductive impairment in mustelids. Guertin et al. (2010) applied an individual based approach by combining fecal DNA genotyping with contaminant and diet analysis of individual river otter scat samples from the Victoria Harbour population. This approach revealed variation in individual contaminant exposure across the landscape along a gradient from industrialized to natural near shore environments. Genetic data also indicated small scale population structuring, suggesting that only certain otters (one subpopulation) were being exposed to contaminants.

This study combined live animal sampling and radio-telemetry with non-invasive sampling. The objective was to evaluate the reliability of individual, population and contaminant data derived from river otter scat. This approach was also well suited for defining individual home ranges and characterizing contaminant exposure in this population. In this study, 17 individual river otters were radio tracked to investigate home range, distribution and movement patterns. Telemetry data reveals that individual river otters inhabit home ranges that span approximately 3kms of coastline. Movement patterns indicate that there is limited if any mixing between the proposed subpopulations and that certain otters inhabit the contaminated areas year round. Understanding the movement patterns and ranges of these individuals will help to further investigate bioaccumulation of contaminants from prey to predator as well as any physiological effects from this exposure. The data derived from feces will be compared to data derived from river otter blood and tissue to investigate bioaccumulation of contaminants from prey to predator as well as any physiological effects from this exposure.

ET05-9

Anticoagulant rodenticides in New Zealand birds - so what?

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The focus for monitoring residual concentrations of anticoagulant rodenticides in wildlife has largely been on birds of prey and mammalian predators. Here we report the results of such monitoring undertaken in New Zealand (NZ) during 2009-2010, predominantly of harrier hawks (Circus approximans) but including other bird species. Concentrations of multiple coumarin anticoagulants (brodifacoum, bromadiolone, flocoumafen coomtarylal) and warfarin were measured in liver tissue using a HPLC method. Residues of at least one anticoagulant were detected in 22 out of 27 harrier hawks. Three hawks had one anticoagulant only, and about half (13 of 27) had two anticoagulants present, most commonly bromadiolone and flocoumafen. Three anticoagulants were present in four of the 27 hawks, and four anticoagulants present in another two cases. Overall, bromadiolone (n=18) and flocoumafen (n=17) were the most frequently detected.

These data add to the growing literature describing the prevalence of anticoagulant residues in non-target wildlife, and also highlight the recurrent question of ‘so what?’ We propose that a laboratory trial using a model bird species and repeated dietary exposure to anticoagulants would be an appropriate first tier step in addressing this question. The aim would be to characterise levels of harm, describe how liver concentrations correspond to risk of mortality and determine the repeated exposure patterns that could ultimately produce a mortality ‘tipping point’ for individuals.

ET06-8

Incidence of poisoning by anticoagulant rodenticides in non-target wildlife in Spain

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Anticoagulant rodenticides (ARs) levels were studied in 401 dead wild and domestic animals in Spain with evidences of AR poisoning, including 2 species of reptiles (n=2), 42 species of birds (n=271) and 18 species of mammals (n=128). Baits (n=32) were also analyzed to detect the potential use of ARs in their intentional preparation. AR residues were detected in 82.0% of the studied animals and 34.9% may have died by AR poisoning according to the clinical information, necropsy findings, residue levels and results of other toxicological analysis. Animals considered with sublethal AR exposure had total AR residues levels (geometric mean 0.13 ppm) significantly lower than those with post mortem findings of secondary AR exposure, especially to second generation ARs (SGARs). On the other hand, granivorous birds showed the highest prevalences of primary AR exposure (51%), especially to chlorophacinone in a region treated against a vole population peak in 2007. The presence of haemorrhages was significantly associated with AR levels in birds, but some animals (7.2%) with elevated residue levels (>0.2 ppm) showed no evidence of macroscopic bleeding. Previous toxicological studies have found a prevalence of up to 3% of cases of poisoning within naturally occurring populations of non-target species. Further current monitoring studies of AR in particular species of wildlife in Spain are also discussed. The use of cumulative SGARs and the application of baits on surface (i.e. treated grain by spreader machines) should be discontinued in future EU regulations on the use of rodenticides to prevent the poisoning of non-target wildlife species.

ET07-9

Global climate change: implications for environmental toxicology and chemistry

ET08-1

Global climate change and influence on chemical fate and bioavailability

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ETD0-2 A pathway-based approach to predicting interactions between chemical and non-chemical stressors: applications to global climate change

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Incorporation of global climate change (GCC) effects into assessments of chemical risk requires an understanding of the effects of mechanisms of chemical toxicity. These effects could include changes in toxicokinetics (chemical absorption, distribution, metabolism and excretion), as well as alterations in toxicodynamic interactions between chemicals and target molecules. GCC can also modify an organism's baseline physiological processes for coping with the external environment (e.g., water balance, thermoregulation, immune-endocrine-neurological systems). In organisms living in GCC modified climates, increased investment in adaptation could lead to heightened susceptibility to chemicals, disease and other stressors. Basically, GCC can cause organisms to be more sensitive to chemical stressors while, alternatively, chemical exposure can make organisms more sensitive to non-chemical (GCC) stressors. Implications of GCC interactions with chemical mechanisms of toxicity are applicable to both ecological and human health effects assessments. To better address the complexities of interactions between chemical and non-chemical stressors, we employed adverse outcome pathways (AOPs) constructs that depict linkages between molecular initiating events and subsequent responses occurring across biological levels of organization, culminating in impacts in individuals or populations that can be used for assessing risk. Through a series of examples and case studies, we demonstrate how chemical- and climate-specific interactions could lead to adverse outcomes. Scenarios are prospective, which project outcomes based on known or anticipated chemical/GCC interactions, as well as retrospective, where mechanisms are proposed for known or demonstrated chemical-climate interactions. Understanding GCC interactions along AOPs provides opportunities for extrapolation across species and biological levels of organization, and between different exposure scenarios, facilitating development of hypotheses and focal areas for further research, and improved inputs for risk assessments.

ETD0-3 Combined impacts of global climate change and toxicants on populations and communities


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SETAC convened a workshop in July 2011, organized into 6 workgroups, to address a variety of issues associated with the potential impacts of global climate change (GCC) on chemical fate, exposure, toxicity and risk assessment. Climatic changes such as higher temperature and CO2 levels have documented negative impacts on many species, e.g., amphibians and coral reefs. GCC-related stress and toxicant exposure can therefore be regarded as multiple stressors, where the combined effects can result in synergistic or antagonistic effects. Moreover, GCC can also independently impact on population and community processes, e.g., disrupt the timing of predator-prey interactions. A great challenge for ecological risk assessment is to predict the how toxicants effects at the individual level (e.g., reduced survival) will be transferred to the population level (e.g., population growth rate) or community level (e.g., species richness). Given the large complexity of direct and indirect GCC impacts on the environment, it is not possible to give general predictions for combined climatic and toxicant impacts and toxicant exposure will propagate from the individual to higher ecological levels. Our approach in this paper, therefore, is to describe relevant ecological mechanisms that will influence the responses of ecosystems to toxicant stress under climate change. In particular we will focus on these topics: (1) Resistance, resilience and recovery from disturbances; (2) Acquired tolerance to stressors and associated costs; (3) Species traits and vulnerability in a landscape context. Within this framework, we use case studies from various aquatic ecosystems to illustrate the complexity of joint effects of GCC and toxicants on populations and communities.

ETD0-4 How will global climate change affect human health risk assessment?

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Global climate change is predicted to alter long-term weather characteristics in different regions. These changes, including increased temperature, greater precipitation extremes, and loss of glacial and polar ice, have implications for human exposure to chemical contaminants. Climate change may also directly and indirectly affect the vulnerability of exposed individuals to chemical exposures. Changes in weather are expected to alter the timing and frequency of use, inputs, fate and transport of chemicals due to climatic and other drivers. Human vulnerability may be affected directly by heat and other weather-related stressors, or indirectly through altered co-exposures or disease patterns. To further explore the implications of climate change for the assessment and management of chemical risks, the authors examine four specific risk contexts: natural toxins, pesticides, air pollutants, and legacy chemicals (e.g., mercury, POPs). For the specific types of decisions to be made in each of these contexts, we examine how assessments and management decisions may be affected by climate change, and how significant the impacts of climate change may be. Climate change is likely to both increase and decrease human exposures, depending on the specific contaminant and specific region or other exposure context. There is limited evidence that climate change will increase the sensitivity of humans to chemical exposures. But small changes in exposure variability or human vulnerability can translate into significant changes in population risk profiles. To assess and manage climate risks effectively, exposure data sources will need to be regularly updated and defaults and assumptions used in exposure assessment evaluated in the context of changing climate. Monitoring and sampling should be done with frequency sufficient to capture variability, which is likely to increase in many places. There are many research gaps in interactions between climate and weather parameters and human responses to chemical exposures. These factors will all exacerbate gaps in chemical protection between developed and developing countries.

ETD0-5 Ecological risk assessment in the context of global climate change


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This paper originates from the SETAC Pellston Workshop entitled “The Influence of Global Climate Change (GCC) on the Scientific Foundations and Applications of Environmental Toxicology and Chemistry” and specifically, from the workgroup charged with determining how ecological risk assessment (ERA) will encompass the
challenges presented by GCC. Although the basic ERA structure remains useful, the process needs to broaden beyond contaminant issues per se to include interactions with changing habitats that alter biotic communities and recognize that landscape ecology needs to be embraced to optimize effective environmental management decisions in the context of GCC. We propose seven principles for conducting ERAs under GCC: 1) evaluate a priori whether climate-related factors are likely to impact to a given ERA process and management decisions; 2) develop and express assessment endpoints as ecosystem services; 3) recognize that responses, (changes in ecosystem services), can be positive or negative; 4) recognize that the risk assessment process requires a more stressor approach with non-linear interactions; 5) develop conceptual cause-effect diagrams that consider context-dependent management decisions, at the appropriate spatial and temporal scales while ranking direct and indirect effects; 6) determine the major drivers of uncertainty by estimating and bounding stochastic uncertainty spatially, and across time; and, 7) plan for adaptive management to account for changing environmental conditions and consequent changes to ecosystem services. Given the complexities and uncertainties associated with GCC, good communication is essential for making risk-related information understandable and useful for managers and stakeholders.

ET10-6
Environmental contaminants and global climate change: implications for environmental damage assessment and restoration/rehabilitation
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ET10A-2
Pollutants and risk assessment parameters are often regarded as random variables in a statistical approach. Beyond statistics, however, toxicological root genes and regulatory components of the genetic variability of net reproductive rate (R0) in a natural Daphnia magna population exposed to Cd stress at two different temperatures. To this end, life history traits were correlated with 20 parental and 19 offspring clonal lineages following a 2(4-squared) design with Cd concentration (control vs. 3.7 µg Cd/L) and temperature (20°C vs. 24°C) as the factors. Offspring lineages were obtained through inter-clonal crossing of the different parental lineages. The population mean, additive and non-additive genetic components of variation in each treatment were estimated by fitting an Animal Model to the observed R0 values using restricted maximum likelihood estimation (REML). From those estimates narrow-sense heritabilities (h2) of R0 were calculated. Significant values of h2 (=0.23) were only found in the 24°C + 3.7 µg Cd/L treatment, suggesting that the ability to produce more offspring under this stressful condition may be inherited across sexual generations. In the three other treatments these values were all low (h2 range: 0.04-0.06) and not significantly higher. Collectively our data indicate that both the sexuual and sexual reproduction phases in cyclic parthenogenetic D. magna populations may play a role in the long-term adaptive potential of Daphnia populations to chemical stress (with Cd as the current example) and that environmental variables other than the chemical itself may influence the adaptive potential to that chemical (with temperature as the current example).

ET10A-3
Do pesticides influence evolutionary processes in natural populations of non-target species? A study in the freshwater snail Lymnaea stagnalis
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Pollutants present in the environment may affect the evolution of natural populations in several ways. Fitness reduction can result from direct effects on the germ-line (mutagenic compounds), or it may be the consequence of a negative impact on genetic diversity, through directional selection (when the population becomes locally adapted) or through amplification of random genetic drift (when local demography and dispersal patterns are impaired). Pollutants may thus affect the evolution of genes under selection as well as neutral regions of the genome. The issue of evolutionary impact is therefore conceptually important for ecotoxicologists and also for ecological risk assessment and management.

With respect to human induced pollutants, some conditions are expected to increase the risk of genetic change in natural populations. Among such conditions, freshwater lentic habitats located within agricultural landscapes are likely to be exposed to recurrent contamination by pesticides, through various modes of transfer from the treated parcels. Non-target species occupying these habitats are thus exposed to a high risk of evolutionary impact, especially when they have low dispersal ability (e.g., when the whole life cycle is aquatic) or opportunity (weak connectivity among occupied sites, e.g., marshes or ditches). The aim of this study was to test the hypothesis that chronic exposure to pesticide cocktails had a selective effect on the studied populations. The applicability of the presented approach to ecological risk assessment will be discussed.

ET10A-1
How evolutionary concepts may enhance ecotoxicology: tracing the genetic background of differential cadmium sensitivities in invertebrates
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Species sensitivity towards pollutants and risk assessment parameters are often regarded as random variables in a statistical approach. Beyond statistics, however, toxicological roots genes and regulatory components of the genetic variability of net reproductive rate (R0) in a natural Daphnia magna population exposed to Cd stress at two different temperatures. To this end, life history traits were correlated with 20 parental and 19 offspring clonal lineages following a 2(4-squared) design with Cd concentration (control vs. 3.7 µg Cd/L) and temperature (20°C vs. 24°C) as the factors. Offspring lineages were obtained through inter-clonal crossing of the different parental lineages. The population mean, additive and non-additive genetic components of variation in each treatment were estimated by fitting an Animal Model to the observed R0 values using restricted maximum likelihood estimation (REML). From those estimates narrow-sense heritabilities (h2) of R0 were calculated. Significant values of h2 (=0.23) were only found in the 24°C + 3.7 µg Cd/L treatment, suggesting that the ability to produce more offspring under this stressful condition may be inherited across sexual generations. In the three other treatments these values were all low (h2 range: 0.04-0.06) and not significantly higher. Collectively our data indicate that both the sexuual and sexual reproduction phases in cyclic parthenogenetic D. magna populations may play a role in the long-term adaptive potential of Daphnia populations to chemical stress (with Cd as the current example) and that environmental variables other than the chemical itself may influence the adaptive potential to that chemical (with temperature as the current example).

ET09-6
Pollutants present in the environment may affect the evolution of natural populations in several ways. Fitness reduction can result from direct effects on the germ-line (mutagenic compounds), or it may be the consequence of a negative impact on genetic diversity, through directional selection (when the population becomes locally adapted) or through amplification of random genetic drift (when local demography and dispersal patterns are impaired). Pollutants may thus affect the evolution of genes under selection as well as neutral regions of the genome. The issue of evolutionary impact is therefore conceptually important for ecotoxicologists and also for ecological risk assessment and management.

Selection as well as neutral regions of the genome. The issue of evolutionary impact is therefore conceptually important for ecotoxicologists and also for ecological risk assessment and management .
Rapid evolution in a Caenorhabditis elegans (nematodes) population: evaluation of resistance costs

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Anthropogenic disturbances affect more and more the evolution of contemporary populations. Pollution, one of the anthropogenic stress, amplifies the intensity of selection pressures which occur in natural populations. Consequently, micro-evolutionary changes may occur very quickly in response to these new forces. Environment can usually underdetermine the genetic changes that will occur in various condition in which this will likely vary with the increase in human activities. These changes may accentuate stress, affecting the life, the growth and the reproduction of individuals, with strong demographic and longer-term phenotypic and genetic consequences.

We started knowing the evolutionary response of a population to changes in one environmental parameter. However, much less is known on the evolutionary reactions of a population to more realistic, multi-variate changes in environmental conditions. We therefore investigated firstly the evolutionary responses of Caenorhabditis elegans populations either exposed to salt (NaCl) or to salt + sodium acetate (NaCl + acetate), and secondly the evolution of reproductive parameters under the selection regime. In parallel, common garden experiments in control confirmed resistance costs associated to genetic divergence. Indeed individual fitness was lower from NaCl and U-populations. Moreover we conducted a reciprocal transplant experiment with U and NaCl from the generation 18. We found resistance costs of the primary selection regime in other stress environment. NaCl-populations reduced their brood size in U compared to control-populations, but it is not true for U-populations in NaCl conditions. Consequently, there can be an addition of new stress that populations suffered and the action of past selection regime on the reduction of genetic diversity. However, we still need to understand the selection mechanisms.
Multi-generational exposure of Folsomia candida to Cd: survival, reproduction and metalloprotein gene effects
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Few studies are currently available on multi-generational effects, particularly with soil invertebrates. In the present study, the effects of cadmium multigenerational exposure were studied in Folsomia candida. Organisms were exposed consecutively to cadmium [EC10 and EC50] along 23 generations of 28 days. Assessed endpoints were survival, reproduction and size. Additionally, the expression level of the metallothionein coding gene has been measured by quantitative real time PCR. Continuous exposure to a concentration of 32 mg Cd/kg (EC10) caused an improvement in performance (total number of juveniles) until F6, after which it started to decline and failed at the 12th generation. Continuous exposure to a concentration of 60mg/kg (EC50) caused an improvement in performance (total number of juveniles) until F6, after which it started to decline but continued at lower level. Population changes could be related to the Mt induction, as continuous exposure to Cd triggered Mt induction which corresponded to reproduction in higher numbers. Interestingly, the higher tolerance observed in the population exposed to the EC50 in comparison to the one exposed to the EC10 could be related with the higher Mt induction levels which may confer larger tolerance and longevity to the particular Further studies are ongoing.

Global DNA methylation in Daphnia magna is influenced by genotype and a wide variety of environmental stressors
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Epigenetics is the study of mitotically or meiotically heritable changes in gene function that occur without a change in the DNA sequence. An important epigenetic control mechanism is DNA methylation, which was recently discovered in the water flea Daphnia magna, an ecotoxicological model species. It has been shown that exposure to relatively high concentrations of certain chemicals can alter the degree of global DNA methylation in D. magna and that this altered DNA methylation level is still found in two subsequent unexposed generations. Here we investigated the impact of a range of environmentally relevant stressors on global DNA methylation. D. magna of 14 days old from two different clones were exposed for 48 h to one of the following stressors: chemical predation cues, strains of the cyanobacteria Microcystis aeruginosa, nutritional quality (essential fatty acids & sterol content), heat stress, metals (166 µg/L Pb, 6 µg/L Cd, 1000 µg/L arsenite) or salinity (5 g/L NaCl increase). All exposures were conducted in triplicate exposure aquaria, resulting in three biologically independent replicates per treatment. DNA was enzymatically digested to nucleosides (including 5-methyl-2′ deoxycytidine), which were analysed by UPLC-MS/MS. Overall, the global degree of cytosine methylation varied between 0.17 % and 2.54 %. An unexpected but interesting observation was the relatively high variation in global DNA methylation level between triplicates of some treatments, with relative standard deviations (RSDs) up to 71 %. The extent of this variation between replicates differed between the various stress exposures, with fish predation cues inducing the largest RSD for both clones. Significant differences in global DNA methylation between treatments were observed. For instance we found 0.83 % cytosine methylation in clone Xinb3 daphnids exposed to Troops medium, which is significantly higher than 0.35 % and 0.44 % in Xinb1 daphnids exposed to salinity or microcystin-producing Microcystis, respectively. Finally, differences between the two clones were observed, with global DNA methylation in the Xinb3 clone generally higher than in the Xinb1 clone, except for daphnids exposed to higher temperature. In conclusion, this study demonstrated that global DNA methylation in D. magna is dependent on genetic (clone) and environmental (exposure) factors. Further research is aimed at addressing the biological function of DNA methylation in D. magna and its potential long-term impact.

ET11A1 Using an integrated assessment framework for contaminants and biological effects to determine good environmental status under the Marine Strategy Framework Directive
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The use of biological effects tools offer enormous potential to meet the challenges outlined by the European Union Marine Strategy Framework Directive (MSFD) whereby Member States are required to develop a robust set of tools for defining eleven qualitative descriptors of Good Environmental Status (GES), such as demonstrating that “Concentrations of contaminants are at levels not giving rise to pollution effects” (GES Descriptor 8). Here we discuss the combined approach of monitoring chemical contaminant levels, along-side biological effect measurements relating to the effect of pollutants, for undertaking assessments of GES across European marine regions. Using data collected as part of the UK’s Clean Seas Environmental Monitoring Programme (CSEMP) we outline the minimum standards that biological effects tools should meet if they are to be used in defining GES in relation to Descriptor 8. Adopting the recommendations of the ICES Study Group for the Integrated Monitoring of Contaminants and Biological Effects (SGIMC) we present a case study demonstrating how such an approach, using contaminant (e.g. metals, PAHs, PCBs) and biological effects (e.g. EROD, bile and pathology) data, in sediment, water and biota could be used to define GES in the marine environment.

ET11A2 PAH and biomarker measurements in fish from condition monitoring in Norwegian waters from 2005 to 2011
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The use of biological effects tools offer enormous potential to meet the challenges outlined by the European Union Marine Strategy Framework Directive (MSFD) whereby Member States are required to develop a robust set of tools for defining eleven qualitative descriptors of Good Environmental Status (GES), such as demonstrating that “Concentrations of contaminants are at levels not giving rise to pollution effects” (GES Descriptor 8). Here we discuss the combined approach of monitoring chemical contaminant levels, along-side biological effect measurements relating to the effect of pollutants, for undertaking assessments of GES across European marine regions. Using data collected as part of the UK’s Clean Seas Environmental Monitoring Programme (CSEMP) we outline the minimum standards that biological effects tools should meet if they are to be used in defining GES in relation to Descriptor 8. Adopting the recommendations of the ICES Study Group for the Integrated Monitoring of Contaminants and Biological Effects (SGIMC) we present a case study demonstrating how such an approach, using contaminant (e.g. metals, PAHs, PCBs) and biological effects (e.g. EROD, bile and pathology) data, in sediment, water and biota could be used to define GES in the marine environment.
Condition monitoring in fish from open seas are performed in Norway every third year. The objectives have been to investigate whether fish from Norwegian seas contain elevated levels of contaminants and to describe the dynamic that occur over the Norwegian continental shelf. The study has been carried out since 2001, with the aim to understand and describe the impact of anthropogenic activities on the marine environment. The study has been carried out in collaboration with the Norwegian University of Science and Technology and the University of Bergen.

In the study, fish from different regions in Norway were sampled and analyzed for a wide range of contaminants, including polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polycyclic aromatic hydrocarbons (PAHs), and heavy metals.

The study has shown that the levels of contaminants are generally lower in the Norwegian marine environment compared to other parts of the world. However, some regions, such as the Barents Sea, have higher levels of some contaminants, particularly PCBs and PAHs.

The study has also shown that there are clear differences in the levels of contaminants between different regions in Norway, with some areas having higher levels of contaminants than others.

The study has important implications for the management of the Norwegian marine environment and the protection of marine species. The results provide valuable information for the formulation of policies and strategies to reduce the levels of contaminants in the marine environment.
ET11B-1

Automatic monitoring of chemical pollution in marine water: opportunities for cost effective solutions

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The European Union has recently established the Marine Strategy Framework Directive (MSFD, 2008/56/EC). The directive foresees the introduction of Environmental Quality Standards (EQS) in territorial waters (roughly 12 nautical miles from the coast line) for a range of chemicals, with particular focus on organic contaminants. Signatory countries are thus required by law to gain adequate infrastructures and tools to perform monitoring and demonstrate fulfillment of the standards. Measurements of organic micropollutants in marine surface waters are challenging and expensive, and often require high logistic costs, for example in relationship to ship hiring and mooring in deep waters.

We present here a new device which allow fully automatic monitoring of priority chemicals using regular line ferries or other fixed stations such as, for example, off-shore platforms. The system allows spatial and temporal integration of the monitoring data, and can be controlled from any computer or mobile device in any part of the world through internet and satellite communications. In addition, the sampler provides automatic procedures for preserving the samples after the collection is completed. Sampling performance is currently under testing both on land based marine stations and on board of a ferry. We present here, in details, the features of the new device and the preliminary results from the proof of concept phase.

ET11B-2

Using ecotoxicological information in an integrative framework for the objective classification of ecological status of marine water bodies

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The European Water Framework Directive (WFD) introduced a new philosophy in environmental management and an ambitious goal: achieving a “good ecological status” of all surface water bodies for 2015. The classification of ecological status relies on setting arbitrary, type-specific reference values for a closed list of biological and chemical quality elements, among which neither biomarkers nor bioassays are considered. Due to natural variability of the WFD-proposed biological quality indicators, multiple problems have surfaced as soon as comprehensive efforts to standardise and harmonise those indicators have started, which is delaying marine water body evaluations beyond the deadlines established in the Directive. As an example, the transitional water bodies of the Minho estuary, the main estuary in the NW Iberian Peninsula, have been evaluated by neither the Spanish nor the Portuguese authorities due to the inexistence of appropriate intercalibrated indices.

An alternative methodology for the classification of ecological status using multivariate analysis, is proposed and applied to a pilot study only for the purpose of illustration of the method. The scale of the study was not designed for classification of ecological status of water bodies. This approach does not depend on the arbitrary definition of fixed reference values and ratio-to-reference boundary values, and it is based on the application of non-metric multidimensional scaling (MDS) and cluster analysis to multivariate data sets encompassing chemical, ecotoxicological and biologically toxicity data. A multiyear data set was generated from the investigative monitoring of marine pollution in Ría de Vigo (NW Iberian Peninsula) from 2004 to 2006, conducted in collaboration with the Spanish Institute of Oceanography (IEO) and the University of the Basque Country (EHU), that included water and sediment chemistry, sediment toxicity assessed by the sea-urchin embryo test, mussel bioaccumulation, mussel physiology (SFG), and benthic macrofaunal richness and density. The application of multivariate analyses to a comprehensive ecological data-set allowed objective and robust classification of sampling sites into discrete categories of ecological status in a high productive coastal ecosystem. This approach allowed integration of biomarkers and bioassays in the evaluation of coastal and transitional waterbodies according to the objectives of the European WFD.

ET11B-3

Managing environmental risk in marine coastal systems: development of an innovative expert decision support system

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Following the EU Marine Strategy Framework Directive (MSFD), member States should attain/maintain/restore a Good Environmental Status (GES) in marine areas in the next years. Risk-based frameworks are useful to objectively assess environmental quality on the basis of both chemical and biological investigations. An innovative Expert System has been developed to support decision makers in managing marine coastal areas and, in particular, sediments. The Expert Decision Support System integrates chemical data by considering the toxic pressure due to all contaminants in sediments: concentrations are compared to TELs (threshold effect levels) and a chemical risk index (ChemRI) is calculated applying an additive model to the pollutant mixture. Ecotoxicological high-level endpoints (i.e. survival and reproduction) are used to calculate the ecotoxicological risk index (EtoxRI), while sublethal parameters (i.e. biomarkers) are integrated into the biological stress index (BSI). With the aim to optimize resources, ecotoxicological test selection should follow a 2-tiers framework: where chemical concentrations overcome PEL (probable effect level) high endpoints (i.e. survival and reproduction) on different model organisms should be evaluated, while if contaminants are in the range between TEL and PEL, sublethal parameters should be added to the ecotoxicological battery.

Finally the Sediment Risk Index (SedRI) is calculated by combining ChemRI and EtoxRI. SedRI ranges form 0 (no risk) to 1 (strong risk): comparing SedRI with specific thresholds is possible to rank the risk level of the sediment and consequently to correctly manage it by planning appropriate interventions (e.g. land-filling, remediation, re-use after dredging). SedRI values in the intervals 0.75-1.00 and 0.50-0.75 respectively indicate high and medium risk; SedRI values in the ranges 0.25-0.50 and 0.00-0.25 individuate mild risk and no risk.

The Expert Decision Support System has been applied to integrate data from the scientific bibliography about sediment quality in some estuarine areas of Spain. Sediments are classified in terms of both chemical contamination and ecotoxicity and recommended interventions are indicated for each site. This work was funded by Theme 6 of the EC seventh framework program through the Marine Ecosystem Evolution in a Changing Environment (MEECE No 212085) Collaborative Project.

ET11B-4

Preliminary evaluation of a new tool for assessment of in situ biological exposure and effects in aquatic environments

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This paper summarizes recent activities associated with the development and demonstration of an integrated exposure and effects assessment approach involving multiple, concomitantly-linked in-situ measurements towards improved ecological risk assessment. When conducted properly, in-situ bioassays are a means of reducing uncertainty associated with traditional laboratory-based characterization of contaminated sediments and surface waters [1-3]. In situ exposures can increase realism through reduced sample manipulation and integration of a variety of site-specific factors, which affect contaminant bioavailability and toxicity. Incorporation of passive sampling devices (PSDs) into in situ studies has recently proven valuable as an additional line of evidence towards evaluating contaminant bioavailability [4]. In some situations, in-situ characterization of sites is the only relevant means of accurately assessing exposure and effects. These scenarios include assessment of (1) in-situ based sediment remediation (e.g. reactive amendment) effectiveness; and (2) time varying stressors (e.g., storm water discharges, tidally influenced groundwater seepage, underwater unexploded ordnance/ discarded military munitions, and oil spills).

The Sediment Ecotoxicity Assessment Ring (SEA Ring) is a recently developed, field deployed device capable of housing an array of in situ bioassay chambers, PSDs, and water quality sensors. Exposure chambers are oriented such that various exposure routes (e.g. surficial sediment, sediment-water interface, water column) can be investigated. A research prototype SEA Ring has been successfully deployed and recovered at marine, estuarine, and freshwater sites varying in water depth and hydrology. These studies tested the approach and have identified major modifications to standard laboratory methods using common test species [5-6]. The prototype SEA Ring is currently undergoing a series of refinements to standardize/control in situ exposure conditions, increase user-friendliness, and reduce dependence on divers. An overview of recent use of the SEA Ring, status of refinements to the device/approach, initial validation of new prototype performance, and discussion of efforts towards regulatory acceptance of the approach will be presented.

ET11B-5

DGT-copper flux measurements predict bioaccumulation and toxicity to bivalves in sediments with varying properties

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Many regulatory frameworks for sediment quality assessment include consideration of contaminant bioavailability. Analyses of pore waters or acid-volatile sulfide (AVS)-
maritime species. This might be due to the logistical problems associated with performing the techniques in open sea or in particular conditions. Moreover no authors have solved the fresh blood conservation problems over a long period of survey and none have assessed the biomarkers of genotoxicity on the cryopreserved blood of marine and

In this period, the organochlorine levels in the blubber of stranded striped dolphin specimens resulted statistically higher than the levels of all other periods and the PCB concentration exceeded those estimated to be a threshold level in aquatic mammals for observed effects on reproduction and imm

Embryotoxic and genotoxic effects of pesticides and heavy metals on embryos of Pacific oyster (Crassostrea gigas)

ET11C-4

2Marine Scotland, Aberdeen, United Kingdom

Stenella coeruleoalba

Embryotoxicity in this sentinel species

First development and validation of genotoxicity biomarkers in frozen total blood of Xiphias gladius and Caretta caretta: a novel approach to evaluate genotoxic effects in organisms living in the surface layer (i.e. majority of organisms). The use of inappropriate or inadequate information for assessing metal bioavailability in sediments may vary the effectiveness of legislation controlling the use of TBT on yachts and ships in the marine environment. As a continuation of this assessment, a further survey was conducted between October 2010 and January 2011. Dogwhells were collected from 76 sites around England and Wales and analysed for impoxes (VDSI and RPSI) and data were assessed according to the criteria developed by OSPAR (Oslo Paris Commission). The results show a significant decline in the level of impoxes. In 1992, 100% of sites showed VDSI values above 2, 2010/2011, only 10% of the sites showed VDSI values above 2 and 42% were below 0.3 (OSPAR assessment class A). The data confirm that the legislation brought in by the International Marine Organisation for large ships, and implemented during 2003-2008, has been very effective in reducing the impact of TBT in the marine environment.

ET11C-1

Biomarker responses in the laboratory are confirmed in large scale field study of ten estuaries

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ET11C-2

Embryotoxic and genotoxic effects of pesticides and heavy metals on embryos of Pacific oyster (Crassostrea gigas)


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Although designed for decades to assess marine water quality, less is known about environmental pollutant genotoxicity and the link between genotoxicity and embryotoxicity in this sentinel species. The aim of this study is to assess embryotoxicity and genotoxicity of two dissolved metals copper sulfate and cadmium chloride (Cul, Cd) and two pesticides (metolachlor, irgarol) in Pacific oyster (Crassostrea gigas) larvae and to investigate the relationship between those two endpoints. Embryotoxicity was measured by calculating the percentage of abnormal D-shaped larvae and genotoxicity was evaluated with DNA strand breaks using the comet assay. After 24-h exposure, the percentage of abnormal D-larvae showed a significant increase from 0.1 µg L-1 for Cu (p < 0.05), 10 µg L-1 for Cd (p < 0.05) and 0.01 µg L-1 for both metolachlor and irgarol (p < 0.001) in comparison with the seawater control. Following 16h exposure, significant increases of DNA strand breaks were observed for both metals (from 0.1 µg L-1 and 10 µg L-1 for Cu and Cd respectively) and pesticides (from 0.01 µg L-1 for both metolachlor and irgarol) (p < 0.05). A strong positive relationship between embryotoxicity and genotoxicity was recorded for each tested toxicant (p < 0.001, R² = 0.71-0.88), except for irgarol showing moderated correlation (p = 0.0019, R² = 0.576), which suggests that some of these pollutants (irgarol, metolachlor and copper) can induce larval abnormalities and DNA damage in exposed oysters at environmentally relevant concentrations.

ET11C-3

First development and validation of genotoxicity biomarkers in frozen total blood of Xiphias gladius and Caretta caretta: a novel approach to evaluate genotoxic effects in

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The aim of this study was to develop and validate genotoxicity biomarkers (comet and diffusion assays) on frozen total blood in order to assess the DNA integrity in stranded swordfish (Xiphias gladius) and the loggerhead turtle (Caretta caretta). Only a limited number of studies have been published that use these assays with these or other pelagic marine species. This might be due to the logistical problems associated with performing the techniques in open sea or in particular conditions. Moreover no authors have solved the fresh blood conservation problems over a long period of survey and none have assessed the biomarkers of genotoxicity on the cryopreserved blood of marine and freshwater fish species and reptiles. In the first phase of this work, a methodology using frozen conservation was developed in parallel with the comet assay on the fresh and cryopreserved blood of Dicentrarchus labrax and loggerhead turtles. In the second phase the techniques were validated on the blood of the loggerhead turtle and swordfish. No statistical differences were observed in the DNA fragmentation and apoptotic cells between the fresh and frozen blood of the Dicentrarchus labrax and loggerhead turtle. Regulation of apoptosis at the fresh and frozen blood of the loggerhead turtle. An age-related decrease in DNA fragmentation and an age-related decrease in apoptosis in the loggerhead turtle was observed. The female swordfish showed higher values of DNA damage (p<0.05) and lower values of apoptotic cells than males. This study related a whole blood cryoconservation protocol applied for the first time in an ecotoxicological investigation. This technique led to the evaluation of possible DNA integrity in two species never studied before in this field, such as the loggerhead turtle and swordfish. The findings strongly suggest that comet and diffusion assays in frozen blood can be used to assess genotoxic damage in a very high variety of species, from threatened species to species used for the environmental monitoring of remote areas. In addition, this innovative methodology could be carried out in all field sampling conditions and not only during laboratory experiments as has been the case until now.

ET11C-4

Temporal trend of organochlorine contamination in stranded and free-ranging striped dolphin (Stenella coeruleoalba) specimens in the Mediterranean Sea

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Mediterranean cetaceans are known to accumulate very high levels of xenobiotic contaminants, such as organochlorines (OCs). In addition they are susceptible to effects of these anthropogenic contaminants such as endocrine-disrupting effects, including consequences on reproduction and immunity. In 1990-1992, a severe Morbillivirus infection affected striped dolphins (Stenella coeruleoalba) along the Mediterranean coasts, starting from Spain and progressively reaching France, Italy, Greece and Turkey. In this period, the organochlorine levels in the blubber of stranded striped dolphin specimens resulted statistically higher than the levels of all other periods and the PCB concentration exceeded those estimated to be a threshold level in aquatic mammals for observed effects on reproduction and immunity. However, the precise roles of these xenobiotic contaminants in the epizoothis are unknown. Several hypotheses were formulated in order to explore the potential link between high OC levels and Morbillivirus infection. The aim of this work is to investigate the temporal trend of the levels of certain organochlorine xenobiotics: dichlorodiphenyltrichloroethane (DDT) and its metabolites and polychlorinated biphenyls (PCBs) in subcutaneous blubber of free-ranging and stranded striped dolphin specimens sampled in the Mediterranean area from
ET11C-5  Organochlorine and polychlorinated diphycylethene compounds in the northern Baltic Sea food web

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Polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans were studied in Yellow-legged gull (Larus michahellis) fresh eggs from several Spanish National or National Park. The main source of organic contaminants in Spanish food web is the agricultural practice. These contaminants are accumulated in the liver, the most important organs of the Iberian Peninsula and all of them are declared as Special Protection Area for birds (SPA). Concentration levels for PCDDs and PCDFs ranged between 3-10 pg WHO-TEQ/g lw. For DL-PCBs, high levels were observed with values ranging from 66 to 400 pg WHO-TEQ/g lw. No significant differences were observed between the samples collected during the two year sampling campaigns (2010-2011). In addition, Audouin gull eggs (Larus audouinii) collected from the Ibiza Delta Natural Park were analysed and the results were compared with those obtained in the Yellow-legged gulls (Larus michahellis). The high PCDD/F and DL-PCB levels found in the Audouin gull eggs were attributed to different feeding habits among species and it confirms that the gull diet has an important role in the accumulation of dioxins and related compounds.

ET11C-6

PCDD/Fs and DL-PCB levels in Seagull eggs from natural and National Parks of Spain

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This study explored PCDD/Fs, PCBs; non-dioxin-like PCBs and dioxin-like-PCBs (DL-PCBs), and PBDEs in the Baltic Sea fish; Baltic herring (Clupea harengus), salmon (Salmo salar), sprat (Sprattus sprattus), whitefish (Coregonus lavaretus), and vendace (C. albulina), and their predator Baltic grey seal (Halichoerus grypus). Contaminant profiles demonstrate possible connections in the food web expressing intense predator-prey relationship. The sampling area covered the Gulf of Bothnia in the time period 2002-2007. PCDD/F sums and WHO-PCDD/F-TEQs were highest in salmon and herring, PCDD/Fs in grey seal existed general in low level. PCDD/F concentration in herring cleared a tendency for lower PCDD/F concentrations in Baltic grey seal than in Baltic herring. The most important prey species of grey seal were: abundant PCDD/F congeners in fish were 23478-PCDD and 2378-TCDF. PCDD/F profile in grey seal was different expressing high contribution of 123678-HxCDD and OCDD. The highest PCB sum was detected in grey seal, followed by salmon and herring. A trend was shown when considering of a sum of 6 indicator-PCB congeners (PCB-28/31, -52, -101, -138, -153 and -180) of which -138, -153 and -180 were most dominating ones in grey seal. WHO-PCB-TEQ was in the highest level in salmon, which indicates effective accumulation of toxic PCB congeners in salmon: The sum of the most dioxin-like non-ortho PCBs was overwhelmingly highest in salmon that affects straight to the WHO-TEQ level seen. Also mono-ortho-PCB sum was highest in salmon. PCB (non-dioxin-like PCB and mono-ortho-PCB) profile was very similar between the fish species and also between fish and grey seal. The profiles of 6 indicator-PCBs were similar in fish species, deviating slightly from use of each species. Non-ortho-PCB profiles were similar between the fish species, when grey seal had cleared more PCB-169 and less PCB-77 than fish. Salmon had the highest PBDE level, although grey seal was almost had equal PBDE concentration. The others stayed quite far below these two species. BDE-47 was the dominating non-ortho PCB congener in all studied species: quantitatively its amounts were biggest in salmon (3.8 pg/g ww) and grey seal; especially grey seal from the Bothnian Bay (9.2 pg/g ww). BDE-209, analyzed only from grey seal, did not contribute significantly to the overall PBDE cluster. Toxic load (WHO-TEQ) of grey seal probably greatly results from preferring herring and perhaps salmon in the diet, especially in the Bothnian Bay.
order of magnitude in both test plots. The results of the other sites will be interpreted during the months to come.

**ET12A-2**

From single-species laboratory toxicity tests to assessing effects on soil biodiversity: how far can we jump?

**C.A.M. van Gestel**

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This contribution will review developments in soil ecotoxicology, emphasizing currently available tools and their potential to predict effects on soil biodiversity and soil ecosystem functioning.

In 1984 the first short-term toxicity tests with earthworms and plants were standardized, while tests were already available for determining effects on microbial processes. By early 2000 toxicity tests using sublethal endpoints were standardized for terrestrial plants, enchytraeids, earthworms, Collembola and predatory mites, and new tests developed for soil microorganisms. For earthworms and enchytraeids, avoidance behaviour tests and a bioaccumulation test have been described.

Most standard toxicity tests use single species, except for the ones on soil microorganisms. To assess toxic effects in more realistic settings, micro-ecosystems were developed to allow quantifying effects at the community level, taking into account species interactions. A field test is available for assessing pesticide effects on earthworms that can be combined with a litter bag test.

For reasons of standardization, most toxicity tests with soil invertebrates use artificial soil, or LUFA 2.2 soil as a suitable and natural alternative. The quest for “putting more eco into ecotoxicology” triggered the focus on more ecologically relevant test designs, integrated approaches with responses at different levels of biological organization, taking into account the normal operating range of ecosystems. More complex issues receive attention, including ecological vulnerability, trait-based analysis and effects on functional endpoints (ecosystem services).

Many of the tests developed for assessing the toxicity of single chemicals are also used for assessing contaminated land, together with chemical analysis and field observations (TREND approach). To support field observations and monitoring, sampling methods for soil invertebrates have been standardized. Interpreting results of field sampling is hampered by difficulties to find suitable references. Sampling efforts like the Dutch Biological Soil Quality network may provide useful reference data. Properly predicting effects on ecosystem structure and functioning in field soils will require moving to more complex test systems and further development of trait-based approaches and the ecosystem vulnerability concept. It will also ask attention for long-term effects of low exposures to persistent pollutants, possibly in combination with other stress factors.

**ET12A-4**
The use of a small-scale terrestrial ecosystem to evaluate pesticide mixtures

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Most studies in soil ecotoxicology evaluate the effects of one chemical in one test species using a reference soil, thus here is a need for a more comprehensive, holistic, hierarchical and integrated approach to assess the impacts of chemical pollutants on soil ecosystems. An attempt to accomplish higher “realism” in ecotoxicity evaluation was achieved through a higher tier experiment, using a small-scale terrestrial ecosystem (“STEM”) containing soil from an agricultural field. Effects of the herbicide glyphosate, the insecticide dimethoate and the acaricide spirodiclofen to soil non-target organisms were evaluated using three concentrations: the field dose, i.e. recommended application dose, 5 and 10 times the field dose. The concentrations were chosen based on the predicted environmental concentration (PEC) according to the FOCUS model for each pesticide. Pesticides were applied at the soil surface, and effects were observed after a 28 days of exposure. Earthworms (Eisenia fetida), isopods (Porcellio scaber) and nematodes (Brassica rapa) were used to survey the effects in single and binary combination exposures of the three pesticides. Results of the individual and binary exposures are discussed taking in account pesticide dissipation along exposure period and effects mainly driven by pesticide or due to interactions occurring in the soil system. In conclusion, the work made with the STEM provided a good insight about the effects of pesticide mixtures to non-target soil organisms, both in functional and structural endpoints, and permitted an evaluation of the interactions between organisms at different trophic levels after exposure to pesticides.

**ET12A-5**
Assessment of the interaction between Cu and temperature changes - a multispecies approach

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Temperature is an important aspect in the study of effects of climate change. Temperature may e.g. affect the reproduction, growth or survival of species. If combined with polluting temperature effects may be even more severe to ecosystems. In order to assess the effects of the temperature alone and in combination with copper pollution in soil population and its processes, a two factorial soil multi-species experiment was performed. Six species representatives of different functional groups were tested over three exposure duration. Feeding activity and OM breakdown were also assessed. A range of temperatures resembling the annual variation for Denmark was used. Results showed that the food-web was significantly affected by Cu pollution and temperature increase. Different species reacted very differently to the distinct treatments applied but the major changes in the abundance of species were in general, attributed to the first 28 days of exposure. Litterbags and bait laminas showed Cu and temperature effects on the OM breakdown.

**ET12A-6**
Evaluation of the risk for soil organisms under real conditions

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Temperature is an important aspect in the study of effects of climate change. Temperature may e.g. affect the reproduction, growth or survival of species. If combined with polluting temperature effects may be even more severe to ecosystems. In order to assess the effects of the temperature alone and in combination with copper pollution in soil population and its processes, a two factorial soil multi-species experiment was performed. Six species representatives of different functional groups were tested over three exposure duration. Feeding activity and OM breakdown were also assessed. A range of temperatures resembling the annual variation for Denmark was used. Results showed that the food-web was significantly affected by Cu pollution and temperature increase. Different species reacted very differently to the distinct treatments applied but the major changes in the abundance of species were in general, attributed to the first 28 days of exposure. Litterbags and bait laminas showed Cu and temperature effects on the OM breakdown.

**ET12B-1**

Monitoring microbial diversity in European soils: ongoing projects and challenges

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Soils represent a huge reservoir of biodiversity which varies in terms of taxonomic richness, relative abundance and distribution according to soil types, climatic conditions, vegetation and land uses. The key function supporting ecosystem services as identified in the Millennium Ecosystem Assessment (MEA, 2005) largely depend upon organisms that inhabit the soil. Thus, the design and implementation of a sustainable soil management strategy requires a better knowledge of soil biodiversity. Against this background,
soil biodiversity is subject to various threats. The most serious are caused by anthropogenic activities which can impair soil biodiversity and functioning with negative consequences on ecosystem service delivery, with consequent effects on primary production and soil sustainability. Determining the range of biodiversity and its impact on soil functioning and ecosystem services is therefore a critical challenge which needs to be addressed.

This contribution will give a soil biodiversity overview, with a specific focus on microbial biodiversity, on national (France, The Netherlands, United Kingdom) and European (EcoFINDERS) initiatives which have been undertaken to assess variations of biodiversity at large spatial scales according to soil and climate types and land uses. The EcoFINDERS (EcoFunctional and Biodiversity Indicators in European Soils) project supported by the European Commission was launched in order to gain information on soil biodiversity (including both microorganisms and fauna). This project will result at the scientific level in increasing our knowledge of soil biodiversity and its role in ecosystem services across different soils, climate types and land uses, at the technological level in the standardization of methods and operating procedures for characterizing soil biodiversity and functioning, and the development of bioindicators, at the economic level in the assessment of the added value brought by cost-effective bioindicators, and of cost effectiveness of alternative ecosystem service maintenance policies. The management of such large sampling schemes requires the development of platforms allowing the extraction, storage and analysis of the DNA, such as the GenoSol platform (www2.dijon.inra.fr/plateforme_genosol/).

ET12B-2

Evaluation of soil biodiversity in Germany - Composition and analysis of soil status with regard to the implementation and advancement of the national strategy on biological diversity.


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After contamination of two agricultural soils from Portugal and Brazil, 1Universität Trier, Trier, Germany 2Department of Agricultural Biotechnology, University of Padova, Legnaro (pd), Italy 3Institute of Ecology, Berlin University of Technology, Berlin, Germany 4CESAM, Dep. of Biology, University of Aveiro, P-3810 193, Aveiro, Portugal 5Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Nederland 6Gaiac, Research Institute for Ecosystem Analysis and Assessment, Aachen, Germany 7University of São Paulo, São Carlos, Brazil 8CESAM, Dep. of Chemistry, University of Aveiro P-3810 193, Aveiro, Portugal 9CECT Oekotoxikologie GmbH, Flörsheim, Germany

SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
Collembola were respectively assorted into order/sub-order/cohort and family. Collembolans were still classified according to morphological traits and used as a case-study of trait-based analysis of Collembola data suggested that a shift in the functional composition of the communities occurred due to carbofuran soil contamination and that species adapted to deeper soil layers were more vulnerable to insecticide toxicity. 

**ET12B-1**

**Effects of insecticidal Cry proteins on nematodes - implications for the ecological risk assessment of a stacked Bt-maize variety (MON89034xMON88017)**

- **Saskatchewan Research Council, Saskatoon, sk, Canada**
- **Environment Canada, Ottawa, Canada**

There is an increasing need in Canada for soil toxicity test methods applicable to the boreal forest and northern regions to serve the needs of both industry and government for assessment and site remediation of thousands of industrial sites in these habitats. To meet this need, Environment Canada and the Saskatchewan Research Council are developing a suite of single-species terrestrial toxicity test methods. Environmental and plant soil invertebrate species were selected and brought into culture in the laboratory. Seeds were obtained from Canadian collections or suppliers or were field-collected while candidate invertebrate species were collected through heat-extraction of various boreal reference soils from across Canada. Growth of plants and survival and reproduction of invertebrates was measured in clean reference soils to establish performance measures. Test method development has focused on seven plant species, an oribatid mite species, two collembolan species and two earthworm species. A case study of toxicity testing of a soil impacted by weathered hydrocarbons will be presented and test results indicate that the use of a test battery of both plants and invertebrates was important for a complete assessment of eco-risk at the site.

**ET12C-2**

**Development of a microbial test method suite for assessment of contaminated soil toxicity to indigenous boreal forest microbial communities**

- **J. Rahn**, L.A. Beaudette, J.T. Trevors, H. Lee, R. Scroggins
- **Environment Canada, Ottawa, Canada**
- **University of Guelph, Guelph, Canada**

Ecotoxicological assessment of soils contaminated primarily with petroleum hydrocarbons (PHCs) and/or metals and salt is important for a complete assessment of eco-risk at the site. Remediation. The current challenge facing regulators, assessors, and managers alike is the lack of a framework or process for the derivation of these Tier 2 SSROs. A case study demonstrating the successful application of the Tier 2 Pass/Fail Approach will be presented. A case study where the approach failed will also be presented. A process that is scientifically defensible will be presented to demonstrate how to derive SSROs where the data signal to noise ratio is small.

**ET12C-3**

**Assessment of contaminated soil in the Canadian boreal forest using standardized toxicity tests**

- **Saskatchewan Research Council, Saskatoon, sk, Canada**
- **Environment Canada, Ottawa, Canada**

Focusing on the actual environmental issue - a dredged sediment application on soils and preceding evaluation of their ecotoxicity to prevent possible ecological risks. High volumes of sediments are removed from rivers and ponds routinely. Although they may have a lot of positive properties (e.g., high nutrient levels) there are reasonable suspicions about contaminants present accumulated sediments. Therefore, an assessment scheme is necessary which helps to decide, if the land application will be allowed for dredged sediment. This assessment cannot be based only on chemical analyses and should be accompanied by ecotoxicological testing. In the Czech Republic, novel directive 257/2009 Coll. for the dredged sediments application on agricultural land was ratified to determine the conditions when it is allowed to use sediment on agricultural soils. Innovation of this directive is the inclusion of four contact bioassays into the assessment. The most important question is, which bioassays should be included in the test batteries for the complex material ecotoxicity testing. The no-less important question is if the ecotoxicity bioassays reflect the contamination of the samples or their physico-chemical properties of other factors. The aim of this study was to answer the question about the appropriate battery of ecotoxicity bioassays for the dredged sediments testing and to investigate the driving factors affecting the results of the bioassays. Extended battery of traditional and new soil bioassays as well as tests of eluates was used for testing thirty-six different sediment samples.

**ET12C-5**

**Ecotoxicological assessment of soils contaminated primarily with petroleum hydrocarbons (PHCs) and/or metals and salt**

- **L. Stephenson**, M. Olausson, A. Angell, J. Shrive, M. Zajdlik
- **Stantec Consulting Ltd., Guelph, Canada**
- **Zajdlik & Associates, Rockwood, Canada**

In Canada, soils contaminated with petroleum hydrocarbons are managed on the basis of four hydrocarbon fractions. There are Tier 1 Canada-wide soil standards for each fraction designed to protect ecological receptors exposed via the direct contact exposure pathway. If these standards are exceeded by fraction-specific concentrations in the soil, then the proponent has the option to conduct a Tier 2 ecotoxicological assessment to demonstrate that 1) the exposure pathway can be excluded; 2) PHC residuals are stable and represent minimal risk to soil organisms; or 3) data generated can be used to derive site-specific remedial objective (SSROs). In practice, Tier 2 ecotoxicological assessments are conducted primarily to demonstrate that PHC residuals are stable and represent minimal risk to soil organisms. The data from the toxicity assessment must satisfy criteria established for different land use classes. This is called the Tier 2 Pass/Fail Approach. If the site passes, then no further remediation or action is required and site closure can be obtained when other lines of evidence corroborate minimal risk. However, if the site soils fail to satisfy the criteria, the proponent must select management alternatives to mitigate risk. Alternatively, the data generated from the Tier 2 ecotoxicity assessment can be used to derive site-specific remedial objectives to guide future remediation. The current challenge facing regulators, assessors, and managers alike is the lack of a framework or process for the derivation of these Tier 2 SSROs. A case study demonstrating the successful application of the Tier 2 Pass/Fail Approach will be presented. A case study where the approach failed will also be presented. A process that is scientifically defensible will be presented to demonstrate how to derive SSROs where the data signal to noise ratio is small.

**ET12C-4**

**What does ecotoxicity testing tell us about dredged sediments?**

- **Research Institute for Amelioration and Soil Protection, Prague, Czech Republic**
- **Research Institute for Amelioration and Soil Protection, Prague, Czech Republic**

Research is focused on the actual environmental issue - a dredged sediment application on soils and preceding evaluation of their ecotoxicity to prevent possible ecological risks. High volumes of sediments are removed from rivers and ponds routinely. Although they may have a lot of positive properties (e.g., high nutrient levels) there are reasonable suspicions about contaminants present accumulated sediments. Therefore, an assessment scheme is necessary which helps to decide, if the land application will be allowed for dredged sediment. This assessment cannot be based only on chemical analyses and should be accompanied by ecotoxicological testing. In the Czech Republic, novel directive 257/2009 Coll. for the dredged sediments application on agricultural land was ratified to determine the conditions when it is allowed to use sediment on agricultural soils. Innovation of this directive is the inclusion of four contact bioassays into the assessment. The most important question is, which bioassays should be included in the test batteries for the complex material ecotoxicity testing. The no-less important question is if the ecotoxicity bioassays reflect the contamination of the samples or their physico-chemical properties of other factors. The aim of this study was to answer the question about the appropriate battery of ecotoxicity bioassays for the dredged sediments testing and to investigate the driving factors affecting the results of the bioassays. Extended battery of traditional and new soil bioassays as well as tests of eluates was used for testing thirty-six different sediment samples.

**ET12C-5**

**Eco-restoration of large-scale anthropogenic disturbances in the Boreal Shield: effects of moisture content on zinc availability and toxicity to the mite Oppia nitens and collembolan Folsomia candida**

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- **ECT Oekotoxikologie GmbH, Florsheim, Germany**
- **University of Saskatchewan, Be-ife, Canada**

Colloquially, soil is often the most common form of anthropogenic stress in ecosystems and can pose threats to human health and the environment. Metal toxicity in soil ecosystems affects microbial, fungal and invertebrate populations. These effects are often compounded by other physical stressors like acidification and moisture stresses. The Flin Flon MB, Canada is a prime example of a boreal area experiencing forest dieback and a loss of plant biodiversity associated with mining and smelting activities for metal ores especially copper and zinc but also cadmium and lead. For many years some effort (mainly liming) has yielded moderate output on restoration in some areas, while other areas are recalcitrant. As part of the effort geared towards understanding the factors that might modulate metal toxicity and/or recovery in the area, we assessed the effect of moisture on Zn toxicity to two invertebrates with contrasting uptake routes but occupying similar trophic levels; the oribatid mite Oppia nitens and the collembolan...
Folsomia candida. We singled out Zn after a relative to Cd toxicity model (TGD) proposed by Hopkin and Spurgeon (2001) identified Zn as the metal of most concern in the Flin Flon area. Initial trials to assess the effects of moisture on Zn toxicity using contaminated field soils (with or without organic matter adjustments) were not successful due to complicating effects of soil properties and low reproduction/survival of the invertebrates. We therefore used an artificial soil system and adjusted the moisture contents to 10, 20, 30, 40, 50 and 60% of the maximum water holding capacity of the soil. After four weeks, we assessed availability of the metals using an ion exchange resin membrane fractionation procedure and compared survival and reproduction as well as metal accumulation in the organisms. The results showed that with increased moisture level, there was increased availability of Zn in the substrates and increased accumulation in the insects. This was further confirmed by the result of life-cycle effect for both invertebrate species which showed increased Zn toxicity as moisture level increased. The results indicated that irrespective of invertebrate species involved, increased having a surging effect on Zn toxicity due to the leaching. Therefore, methods used for keeping soil moisture low in remediated areas would be of importance in eco-restoration of contaminated sites. The implications in risk assessment of Zn in contaminated sites are discussed.

ET12c - Applicability of the Caenorhabditis elegans survival, growth and reproduction test to assess the effects of biosolids used in agriculture

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3Modern High productivity agriculture has led to an impoverishment of nutrients and organic carbon in field soils, and as a consequence plants nutrition complementes like fertilizers or wastes have to be used to keep high yield. These complex materials can also be a source of toxicants, depending on their origin, so there is a necessity to assess their environmental effects before field use. In this study, biosolids used in agriculture were tested as a mix with standard soil at different field dose through eco-toxicity test on the nematode Caenorhabditis elegans; endpoints for this organism being survival, growth and reproduction. In order to run these tests we first followed the standardized protocol for sediment and soil testing with C. elegans (ISO 11348, 2010). These tests showed that the testing could be significantly different according to the type of soil (e.g. two phases system with overlaying water). Indeed, soil moistening in the standardized protocol is based on soil dry weight which means that the same amount of water is added for every soil, regardless of the water holding capacity (WHC) of the samples. Moreover, some biosolids can have a high WHC, what increase humidity's differences among soil and soil mixed with biosolids when moistened as recommended by this protocol. To prevent this, improvements of the standardized protocol were developed, in which water addition is based on soil WHC instead of soil dry weight. Moreover, food volume added to the samples was lowered in order to be able to include it in the volume for soil moistening. In this context and in order to validate this new method, comparisons between standardized and optimized protocols were carried out for endpoints responses in five soils with different textures. This optimized protocol was used to assess the effect of different biosolids (limed sludge, manure ...) mixed with standard soil at different field rates and an example for a limed sludge is given. Regarding these results, improvements of the standardized protocol are adapted to assess the effects of these materials on C. elegans.

ET13 - Natural toxins and bioactive compounds

ET13a1 Hydroxylated / Methoxylated analogs of Polybrominated Diphenyl ethers: biotransformation products of PBDEs or natural products

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Brominated compounds are ubiquitous in the aquatic environment. The polybrominated diphenyl ether (PBDE) flame retardants are anthropogenic compounds of concern. Results of some studies have suggested that PBDEs can be biotransformed to hydroxylated brominated diphenyl ethers (OH-BDE) and subsequently to methoxy PBDE (MeO-BDE). However, the efficiency of transformation of PBDE to OH-BDE formation observed has been extremely small and could be accounted for by trace contaminants in the experimental materials. OH-BDEs have also been identified as natural compounds produced by some marine invertebrates. Another class of compounds, the methoxylated BDEs (MeO-BDEs), has also been identified as natural compounds in the marine environment. Both the OH-BDEs and MeO-BDEs bioaccumulate in higher marine organisms. Recent studies have demonstrated that MeO-BDEs can be biotransformed to OH-BDEs and this generates greater amounts of OH-BDEs than could be generated from PBDEs. Consequently, MeO-BDEs likely represent the primary source of metabolically derived OH-BDEs. Given that for some endpoints OH-BDEs often exhibit greater toxicity compared to PBDEs, it is prudent to consider OH-BDEs as chemicals of concern, despite their seemingly "natural" origins.

ET13a2 Release and distribution of the bioactive compound artemisinin in the soil in and near Artemisia annua plantations

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Growing of biomedicine crops, i.e. bioactive compounds produced in plants and extracted for pharmaceutical products, require moncultures of plants with high contents of the desired bio-active molecules. Artemisin is produced by the mosquito repellant species Artemisia annua L. and used as active ingredient in anti-malaria drugs. Cultivation of A. annua in field scale implies high amounts of artemisinin produced and potential high losses to soil and leaching to the aquatic environment, where artemisinin could impact vulnerable organisms. Knowledge of the release routes of active compound from plant to soil, soil concentrations as well as within and around plantations is pivotal to risk assessments of biomedicine plantations. To evaluate the release routes A. annua was grown in the green house, where leaf content, total soil content as well as contributions to total soil content from debris of dead leaves, rain, and root exudation of artemisinin were measured every second week over a full growing season. The dynamics of artemisinin in a field soil was investigated under different conditions measuring artemisinin in depth integrated samples (0-100 cm) and along a transect (0.20 m in the plantation). Artemisin was measurable up to 15 ms away from the A. annua plantation in the direction of the dominating wind. Large amounts of precipitation during the experiment facilitated transport of artemisin to lower soil layers at the level of the drainage pipes. Hence, leaching of artemisinin to streams and lakes is a considerable risk. Rain fall contributed to the release of artemisinin to the soil environment, but the biggest contributor was dead leaves. Root exudation was a minor contributor. The amount of artemisinin followed the leaf contribution, whereas the total concentration in soil followed the amount released from dead leaves. The artemisinin content in soil reached steady state around week 21, suggesting a degradation rate in the similar magnitude as the input rate. A model of inputs and outputs of artemisinin to the soil environment will be provided at the symposium, as a tool for risk assessment and risk managing when cultivating plants producing a bioactive compound.

ET13a3 Production and emission of mycotoxins from a Fusarium infected winter wheat test field

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3Mycotoxins are naturally occurring secondary metabolites of fungi colonizing a variety of cereals, fruits, vegetables and organic material in the soil, but can also arise due to moist conditions during storage. Mycotoxins have been studied intensively for decades due to their occurrence in food and feed and, hence, their potential threat to human and animal health. Recently published data on two prominent mycotoxins, i.e., deoxynivalenol (DON) and zearalenone (ZON), indicates that the aquatic environment can also be exposed to mycotoxins. The mycotoxins in this study were measured in wheat from the infection experiment using a culture of the Fusarium graminearum, which was grown in a field experiment in the Folsomia candida. We singled out Zn after a relative to Cd toxicity model (TGD) proposed by Hopkin and Spurgeon (2001) identified Zn as the metal of most concern in the Flin Flon area. Initial trials to assess the effects of moisture on Zn toxicity using contaminated field soils (with or without organic matter adjustments) were not successful due to complicating effects of soil properties and low reproduction/survival of the invertebrates. We therefore used an artificial soil system and adjusted the moisture contents to 10, 20, 30, 40, 50 and 60% of the maximum water holding capacity of the soil. After four weeks, we assessed availability of the metals using an ion exchange resin membrane fractionation procedure and compared survival and reproduction as well as metal accumulation in the organisms. The results showed that with increased moisture level, there was increased availability of Zn in the substrates and increased accumulation in the insects. This was further confirmed by the result of life-cycle effect for both invertebrate species which showed increased Zn toxicity as moisture level increased. The results indicated that irrespective of invertebrate species involved, increased having a surging effect on Zn toxicity due to the leaching. Therefore, methods used for keeping soil moisture low in remediated areas would be of importance in eco-restoration of contaminated sites. The implications in risk assessment of Zn in contaminated sites are discussed.

ET13a4 Risk assessment of Bacillus thuringiensis var. israelensis and spinosad on Polypedilum nubifer and Tanatarus curticornis (Diptera: Chironomidae) in coastal wetlands

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3Larvicides used for mosquito control are introduced into aquatic ecosystems where mosquito larvae develop (marshes, ponds, sanitation devices). A number of natural products of them exhibit selectivity towards certain insect taxa which promotes their use for mosquito control. This is the case for the bacterial larvicide Bacillus thuringiensis var. israelensis (Bti), widely used for mosquito control all over the world, and for Spinosad, a mixture of spinosyns A and D known as fermentation products of a soil bacterium. Therefore, the present study was undertaken to assess the impact of Bti and spinosad on Mediterranean coastal wetland populations of Polypedilum nubifer (Skuse) and Tanatarus curticornis Kieffer (Diptera: Chironomidae). Unlike Bti, spinosad has a strong lethal effect on P. nubifer population and seems to affect T. curticornis at presumed recommended rates for field application. But differences observed in term of sensibility...
between this two chironomid populations confirm that the assemblage of the arthropod community as well as the population dynamic need to be known for the evaluation of risk of pesticides at the population level.

ET13A-5

New patchwork of toxicity: impact on marine ecosystems
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This work was done under the project ALTANTOX, aiming the discovery of emerging marine toxins in the Atlantic ocean, increased by the impact of anthropogenic inputs and climate change. For that purpose the Portuguese coast was surveyed between 2009 and 2010 for three distinct biotoxins (Saxitoxin group (PSP), Spiroliudes (SPX) and Okaacidic Acid (OA)) in 15 bentic species of molluscs and echinoderms. These species where selected by their importance in the food-chain and to search for the presence of new vectors. The invertebrates were collected along the Portuguese coast since the summer of 2009 till the end of 2010.

For PSP's samples were analysed by the Lawrence method, for SPX LC-MS/MS technique was performed, and UPLC-MS/MS for OA analyses.

Results show that we are in presence of 16 first reports of these endemnic species: PSP's in Gumbilicalis, N.lapillus, Monodonta sp., P.lividus, M.glacialis and A.deplano; SPX in Gumbilicalis, N.lapillus, Monodonta sp., M.glacialis and Pantermedia; OA in Gumbilicalis, N.lapillus, Monodonta sp., P.lividus and M.glacialis. These results are an important result in an area with no reports. The values obtained for some species such as the sea star and the gastropods N.lapillus show that toxin transfer into food chain occur via mussels. New toxin routes especially including edible ones provide evidences that monitoring of marine toxins should be extended to other species than bivalves in order to prevent human health risks.

ET13A-6

Variations in the microcystin congener composition of lakes
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Cyanoctis can produce a wide range of compounds which are toxic to other organisms including humans. The microcystins appear to be the most widely encountered cyanotoxins in freshwater and over 70 different types of congeners have been described. The drinking water standards are based on microcystin-LR and less attention has been given to other congeners. However, other microcystins and the actual composition observed in lakes should also of be interest since some microcystins are much more toxic than others. Unfortunately only about a dozen microcystins are commercially available and can thus be routinely quantified. At present, the factors that regulate the composition of microcysts in the environment are largely unknown. Culture studies and empirical observations of European freshwater mussels (D. polymorpha and U. tumidus) show that toxin transfer along food chain occur via mussels. New routes especially including edible ones will produce evidences that monitoring of marine toxins should be extended to other species than bivalves in order to prevent human health risks.

ET13B-1

Trade-offs in herbicide-stressed Microcytis aeruginosa: growth vs. toxin production
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Organisms allocate assimilated resources into competing metabolic functions, such as maintenance, growth and defense. This pattern of allocation may change in response to stress or in the increment of resources allocated to one trait and a decrement to another trait. The aim of the study was to test for the existence and magnitude of a potential trade-off between growth and toxicity production in Microcytis aeruginosa exposed to the herbicide glyphosate at different light regimes. The ecological costs of microcystin production were estimated from differences between growth rates in a wild type strain of M. aeruginosa (PCC 7806) and its mutant unable to produce microcystins (MC-LR, 7806). The trade-off (negative fund of brown algae) triggered by glyphosate was detected only when cultures were incubated under photoperiods longer than 8 h of light (12:12 and 16:8), with different patterns for the microcystin analogues, time of incubation, and exposure concentration. Growth was the prioritized trait (ratio >1), especially under the shorter (8 h) and longest photoperiods (16 h) during the first week of exposure. The magnitude of the trade-off ranged from 3-3 [GREETK] for the most toxic microcystin analogue MC-LR and and 2.5-2 [GREETK] for its demethylated variant [D-Asp]MC-LR. The ecological costs involved in microcystin production were higher during the first week of incubation compared with the second. The mutant unable to produce microcystins had generally a higher growth rate than the wild type, regardless of presence or absence of glyphosate and especially during the first week of incubation. Glyphosate conferred additional ecological advantages to the mutants expressed in terms of tolerance and benefits to the wild type cultures during late exponential growth phase by either increasing toxin production or minimizing the extracellular releases to the medium. The negative production rates calculated for [D-Asp]MC-LR during the first week of incubation might imply that the [D-Asp]MC-LR pool is not stable and that the compound is synthesized, used and replenished constantly under the given culturing conditions. Growth, seems to be a conservative trait in M. aeruginosa because is preserved over toxin production which limits its application as ecotoxicological endpoint in risk assessment of pesticides.

Microcystin production seems more responsive (sensitive) to glyphosate in time and at lower concentrations than growth.
and algae, generally more for the compounds excreted to their surroundings. Exudates from two algal species and from most tested pure strains of cyanobacteria elicited estrogenic activity with concentrations of estrogenic equivalents in the range of ng/L, which could be considered relatively large since equivalent potencies of estrogens have been shown to cause reproductive toxicity to aquatic animals. Aqueous extracts caused significant estrogenicity only in case of both algal species and one cyanobacteria (Aphanizomenon gracile) with estrogenic equivalents ranging from 1.5 to 280 ng 17β-estradiol/L. There does not seem to be any clear relation between the estrogen potency and concentration of known cyanotoxins. The research was supported by the Czech Science Foundation grant No. P503/12/0533 and by the project CETOCEON (CZ.1.05/2.1.00/01.0001) from the European Regional Development Fund.

ET13B-4
Seasonal variation of immune parameters in mussels (Ellipitio complanata) exposed to natural bloom of cyanobacteria
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Toxic cyanobacteria can have harmful impacts on aquatic organisms. In Lakes located in the Eastern-township (Québec, Canada), Microcystis aeruginosa blooms drift into littoral areas. We studied the effects of M. aeruginosa on the immune system of a common mussel (Ellipitio complanata) during an algal bloom. In order to verify the toxic potential of these algae, we immersed caged mussels during 4 weeks for three consecutive months, from August to October, in four different sites. Among these sites, three of them were located in the St. Lawrence and the fourth one in the Lac St-Pierre and is considered the cleanest site (LSP). M. aeruginosa bloom occurred in Choinière in September. The immune parameters consisted in haemocyte integrity and phagocytosis were analyzed each month by flow cytometry. Two-way ANOVA (interaction term) indicated that immune parameters exhibited marked temporal variation in different sites. In the LSP site, haemocyte viability remained constant throughout the experiment in Boivin and Choinière but a significant decrease was measured in September and in October in both Yamaska and LSP sites. At Choinière, phagocytosis activity remained relatively constant after each monthly exposure even though a significant decrease was observed in both LSP and Yamaska in September. Mortality of haemocytes could be a sign of cytotoxicity and mechanical or chemical disturbance, which cyanobacteria bloom does not seem to be. Yamaska outlet incorporates all sources of pollution both agricultural and industrial but LSP results are quite surprising as it represents the cleanest site. A decrease of the phagocytic index is usually observed response to in vivo or in vitro exposures to toxic concentration of xenobiotics or a hormesis phenomenon. Consequently, ingestion of algal during a bloom by mussels does not provide evidence of a depressed immune status during a bloom. Furthermore, the present study will investigate other effects of these toxins on the invertebrate thiol production, reactive oxygen species (ROS) production, cytochrome oxygenase (COX) and nitrate production in mussel haemolymph.

ET13B-5
Impact of natural cyanobacterial biomass containing microcystin-LR on larval stages of the amphibian Xenopus laevis
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Cyanobacteria are known to produce several compounds and especially their secondary metabolites, so-called cyanotoxins, might have severe effects on vertebrates according to their chemical structures. The main occurring cyanotoxins, microcystins (MCs), are considered as being potent hepatotoxins causing a threat for health of vertebrates including humans. Despite aquatic vertebrates are primarily exposed to cyanobacteria and especially to cyanotoxins, in their natural habitat, most studies with MCs were performed using non-aquatic vertebrates e.g. mammals. In aquatic vertebrates most experiments have been carried out in fish by unnatural exposures via injections of pure cyanotoxins. In amphibians, only a few data are available to assess MCs impacts during embryonic development. However, only postembryonic larval stages are characterized as the most sensitive period concerning developmental and sexual aberrations. In the present study, tadpoles of the South African clawed frog Xenopus laevis were exposed for 21 days to MC-LR provided with diet as a natural exposure route. Effects of diets containing natural cyanobacterial biomass resulting in considerable amounts of 42.8 and 187.0 µg MC-LR/kg diet, respectively, were investigated concerning bioaccumulation, development, stress and detoxification in order to determine for the first time potential toxicological impacts of cyanobacteria on postembryonic stages of amphibians. The fate of MC-LR at concentrations of taken up via ingestion was determined in whole body using liquid chromatography with tandem mass spectrometry detection. Beside mortality and weight effects on metamorphosis were assessed by recording developmental stages. In parallel, corticosterone levels were determined as stress biomarker. In addition, gene expression of endocrine parameters associated with metamorphosis and sex steroid differentiation as well as decrease of the detoxification enzymes of all three biotransformation phases was assessed. Interestingly, exposure to MC-LR containing cyanobacterial biomass applied via natural food could not affect metamorphosis and only slight impacts on weight and corticosteroid levels were found. It seems likely that X. laevis underwent evolutionary adaptations to cope physiologically with toxic cyanobacteria occurring in the same habitat, enabling survival even at MC-LR concentrations being rather toxic for mammals.

ET14-1
Bringing ecological processes into ecotoxicological risk assessment

ET14-2
Fungicidal effects on a decomposer-detritivore-system
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In heterotrophic streams, leaf litter breakdown is a fundamental process that is primarily realized by decomposing microorganisms, especially fungi, and detritivorous, leaf-shredding macroinvertebrates. Although fungicides, due to their unspecific modes of action, may affect both groups of organisms, up to date no test design exists to assess combined effects (i.e. direct toxicity and indirect effects) on shredders. Therefore, the present study evaluated this scenario using a five-week semi-static test design with Gammarus fossarum as test organism and tebuconazole (65 µg/L) as model fungicide. Hence, gammarids directly exposed to the fungicide additionally received leaves conditioned (i.e. colonized and altered by microorganisms) in the presence of tebuconazole. Hence, gammarids exposed to fungicides produced significantly less faeces and significantly reduced fungal biomass (~40%) and a significantly reduced sporulation (~30%) associated with tebuconazole exposed leaves. However, the significantly reduced lipid content (~20%) of gammarids exposed to the fungicide indicates that combined effects of tebuconazole were not fully compensated by the increase in assimilation. The present study, thus, indicates that fungicides may affect energy processing in gammarids, a key species in leaf litter breakdown. This may eventually translate into alterations in energy transfer in heterotrophic streams. Consequently, test design similar to the one used during the present study - assessing combined effects - should be used in the future to validate the protectiveness of environmental risk assessment for decomposer-detritivore-systems and consequently leaf litter breakdown.

ET14-2
Trophic niche metrics based on stable isotopes (d13C and d15N) for the assessment of the functional effects of toxic substances on aquatic food webs
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Toxic substances may have negative impacts leading to cascading effects on food webs through e.g., changes in the nature and abundance of available food resources or inter-specific relationships. This may lead to effects on various functional processes that ultimately translate into changes in ecosystem properties. The identification of parameters that may provide information on the effects on ecosystem functioning therefore constitute a challenge for the future of ecological risk assessment. Measurements of the natural abundances of stable isotopes of various elements in environmental samples are widely used in ecological studies to characterize the trophic position of species and to identify the sources and pathways of matter across food webs. Various quantitative metrics of trophic niche based on such analysis have recently been proposed, that proved to be efficient in identifying the effects of environmental degradation on aquatic food webs.

In this study the characteristics of the trophic niche of a freshwater snail species, Radix peregra, were characterized in outdoor control and fungicide-exposed artificial streams. Streams (length: 40 m, depth: 50 cm) were exposed to a diithiocarbamate fungicide, thiram. Two concentrations (nominal values: 35 and 170 µg L-1), were used, with two replicates per concentration. Four untreated streams were used as controls. Streams were continuously exposed for three weeks, followed by a two month-long recovery period. Samples of snails and their potential food sources (filamentous algae, biofilm, litter) were collected in the streams just before the beginning and immediately after the end of the exposure period, and at the end of recovery period. Stable carbon (δ13C) and nitrogen (δ15N) isotope signatures of samples were measured using mass spectrometry. The results were used to compare the values of various trophic niche metrics that were compared between control and contaminated systems. The results clearly show a significant effect of thiram on the characteristics of the trophic niche of R. peregra and on their dynamics. Changes in the structure of the benthic food web in the streams were associated with changes in the nature and abundance of food sources used by R. peregra. Stable isotope signature of snail tissues mirrored these changes therefore suggesting that this approach may prove to be useful to assess some of the functional consequences of toxic substances on aquatic food webs.

ET14-3
The do’s and don’ts of putting eco into ecotoxicology

SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
Exposure to TPT came from laboratory experiments. We used a newly developed IBM for the evaluation of the EC50 of Daphnia and the application of an acute effect assessment for insecticides by using threshold concentrations for treatment-related effects as observed in micro/mesocosm studies treated with insecticides.

In conclusion, for species with similar individual level responses to toxicants, population level responses may be vastly different due to the influence of different life-history processes, competition should be taken into account. We found that competition substantially prolongs population recovery after contamination: Without interspecific competition the population of the sensitive species recovered within 9 time steps after a contamination event that killed 50% of the individuals. In contrast to that, recovery time increased to up to 34 time steps in the presence of an insensitive competitor. Furthermore, chronic toxic effects on the individuals that decreased reproduction capacity or survival probability increased the adverse effects on the population level. In particular, species with lower reproduction capacity were strongly affected in the presence of competitors and additional chronic effects. In extreme cases populations of such species eventually went extinct even with low acute and chronic effects of the toxicant on the individuals. We showed the structural realism of the model as it produced comparable pattern of the relationship between the tow competing species abundances as observed in the semi-field study.

Our findings imply that both experimental and modelling studies that do not consider the interaction between interspecific competition and toxicant effects will largely overestimate the speed of recovery processes. The inclusion of species interaction in ecotoxicological models aids a more realistic prediction of long-term effects on the population level from the effects observed in a single-species application.

Using a modelling approach to compare sensitivities to Triphenylbin in the individual and population levels for three planktonic organisms D.P. Kulkarni1, T. Strass1, U. Hommen1, A. Gerger, H.T. Ratte1, T.G. Preuss2

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Triphenylbin (TPT) is persistent in the environment and toxic to aquatic organisms, for assessing effects of chemicals using mesocosms and to include this information in the ecological risk assessment process. The purpose was to bridge the gap between the data gathered for the risk assessment (standard laboratory tests) and the protection goal (ecosystems). In the field of (chemical) stress, ecological processes are used and adapted for inclusion in risk assessment. In this paper we will review the studies that uses species traits and the processes between species traits and the determinants of ecosystem response to chemical stress. The do's and don'ts will be identified and discussed as well as a way forward. Van den Brink (2008) defines vulnerability as a combination of intrinsic sensitivity, recovery potential and ecosystem interactions. Although sensitivity is not an ecological trait its inclusion in the ecological risk assessment of chemicals is pivotal. For the establishment of the relationships between traits and sensitivity or when sensitivity is used in retrospective risk assessment, mode of action specific approaches need to be developed. For a successful use of indices of chemical stress, their specificity should also be proven by assessing their correlation with other types of stress, like sedimentation, nutrient and habitat destruction. Recovery is determined by the sensitivity and life history characteristics, like dispersal ability and reproduction, of the species and the structure of the landscape under consideration. Therefore, population models are ideal tools to estimate of recovery for a particular species in a defined landscape. In order to improve the ecological foundation of risk assessment, there is a great need for modelling and experimental studies that addresses the importance of habitat quality, multiple stress and both intra- and inter-specific interactions for the recovery of affected populations. Micro- or mesocosm experiments are considered a realistic and useful approach to directly assess chemical effects on ecosystem structure. Measurements of ecosystem functioning, however, are much more scarce. Food web models can either be used to assess the effects on ecosystem function from an existing mesocosm data set or can be used for the assessment of chemical risk for a certain ecosystem.

Predicting community effects of toxicants considering interaction with stressors M. A. Beketov1, B. J. Kefford2, R. B. Schafer3, S. Duquesne4

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Environmental risk assessment aims at predicting effects of PPPs on non-target communities so that unacceptable effects are avoided. To do so, a tiered approach is used that ranges from lower tier to higher tier testing, characterised by an increasing realism. Relevant processes to be considered in this context are interactions of toxicants with biotic and abiotic stressors. Such interactions can act in an additive or synergistic manner and the identification of these effects is highly relevant to further improve risk assessment of toxicants. The aim of this contribution is to identify the possibilities and limitations of a systematic prediction of magnitude and direction of toxicant effects when interacting with biotic and abiotic stressors in aquatic ecosystems. We show that interactions of toxicants with biotic and abiotic stressors alter the sensitivity of populations. They can vary up to two orders of magnitude. Ecological traits that are relevant to predict outcomes of interactions were identified. Finally, we suggest a procedure related to the trait based indicator system SPEAR to validate predictions of toxicant interactions with stressors. We conclude that considering interactions with biotic and abiotic stressors greatly enhances the quality of the effect assessment of toxicants.

Competition matters: modelling long-term population level effects of contamination M. Kattwinkel, M. Liess

UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany

Competition matters: modelling long-term population level effects of contamination. We found that competition substantially prolongs population recovery after contamination: Without interspecific competition the population of the sensitive species recovered within 9 time steps after a contamination event that killed 50% of the individuals. In contrast to that, recovery time increased to up to 34 time steps in the presence of an insensitive competitor. Furthermore, chronic toxic effects on the individuals that decreased reproduction capacity or survival probability increased the adverse effects on the population level. In particular, species with lower reproduction capacity were strongly affected in the presence of competitors and additional chronic effects. In extreme cases populations of such species eventually went extinct even with low acute and chronic effects of the toxicant on the individuals. We showed the structural realism of the model as it produced comparable pattern of the relationship between the tow competing species abundances as observed in the semi-field study.

Our findings imply that both experimental and modelling studies that do not consider the interaction between interspecific competition and toxicant effects will largely overestimate the speed of recovery processes. The inclusion of species interaction in ecotoxicological models aids a more realistic prediction of long-term effects on the population level from the effects observed in a single-species application.

Using a modelling approach to compare sensitivities to Triphenylbin in the individual and population levels for three planktonic organisms D.P. Kulkarni1, T. Strass1, U. Hommen1, A. Gerger, H.T. Ratte1, T.G. Preuss2

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by comparison of RACs obtained from the new first-tier data requirements to ecological safe threshold concentrations for arthropods communities as derived from semi-field studies implies that the new data requirements in case of insecticides and using acute toxicity data from D. magna and A. bahia or an OECD-chironomis seem to be protective in most cases and is an improvement compared to the old data requirements.

ET15A-2  
A focal fish species for pesticide risk assessment in the EU
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Extrapolation is needed to link standard toxicity endpoints to protection goals. Ecological modelling can serve as an extrapolation tool from the individual to the population level and from one species to another species. However, it is crucial to decide on a suitable species to be modelled. For this purpose, we established a procedure to identify a focal species, a realistic worst-case which is thus representative of the life histories that might occur in the field, on conservative basis. We adopted the vulnerability principle which consists of (a) intrinsic sensitivity, (b) external exposure and (c) ability to sustain a population. The procedure allows the definition of a focal species assuming that all fish can be intrinsically sensitive to certain levels of plant protection products (PPPs).

Out of 379 fish species living in European freshwaters, 27 were found to be native to Europe, widespread in at least one of the EU regulatory zones of authorization of PPPs, and inhabiting streams, ditches or ponds. Based on the reviewed studies on species assemblages, the presence of these species in agricultural settings was verified, and hence their potential external exposure to PPPs.

Deterministic age-based matrix models (time step 1 year) are being parameterised from published field demographic studies. The procedure was exemplified so far for four species whereby proportional effects of alterations in juvenile survival, adult survival or fertility on population multiplication rates \( \lambda \) were compared. Since fish are protected on the individual level against visible mortality, the most important factor in our analysis is the reduction in fertility and its effects on the population multiplication rate of a species.

The focal species will be identified and presented; a fish species that is native to Europe, widespread in the EU, lives in edge-of-field water bodies and has the lowest ability to sustain its population (highest proportional effect or elasticity of \( \lambda \)) as compared with the other species in case a PPP reduces its fertility and thus compromises this ability.

ET15A-3  
In situ GamTox for assessment of acute and chronic pollution in small streams
A.E. Gerhardt1, M. Koster2, V. Leib3, F.R. Lang4
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2Amt für Umwelt, Fraenzenfeld, Switzerland  
3Amt für Umwelt und Energie, St. gallen, Switzerland  
4Interkantonales Labor, Schaffhausen, Switzerland

In the 1st experiment, we observed only survival while during the 2nd experiment, we also observed feeding rate by weighing the food. G.pulex exposure, and that a strong rebound in feeding activity occurred after exposure. Effects of imidacloprid to the growth and reproduction of D. magna were observed within 1Eawag, Dübendorf, Switzerland

ET15A-4  
Sub-lethal effects of imidacloprid pulses to aquatic invertebrates
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1University of York, York, United Kingdom

Observing behavioural changes of organisms evoked by relatively short and low concentrated stress in ex-situ studies is a useful tool to address xenobiotic impacts in a more environmentally-relevant manner. Ecosystems are usually exposed to low concentrations of toxicants and environmentally-relevant concentrations rarely cause mortality nor are continuously present.

We aimed to contribute to the understanding of the time course of sub-acute effects of short-term exposure to xenobiotics to non-target species by undertaking ex situ studies with a recovery phase. The feeding rate of the shredder Gammarus pulex and a range of sub-acute endpoints for the crustacean Daphnia magna were observed. We measured the influence of the neonicotinoid insecticide imidacloprid to the feeding rate of G. pulex within a feeding activity assay consisting of a four day exposure phase extended with a five day recovery phase. The impact of a one-week exposure of new born D. magna to imidacloprid was observed measuring growth and reproduction subsequent to exposure for approximately four weeks.

We found that concentrations within the range of measured and estimated environmental concentrations caused significant decreases in the feeding activity of G. pulex during exposure, and that a strong rebound in feeding activity occurred after exposure. Effects of imidacloprid to the growth and reproduction of D. magna were observed within and after exposure. Both endpoints were still concentration dependent and significantly affected four weeks after exposure. Inhibition in growth was observed at the lowest concentration tested, whereas inhibition in reproduction was observed at concentrations of at least one order of magnitude higher.

Lethal and sub-terminal endpoints can be still valid beyond the presence of the toxicant. Observing the endpoint of interest above the direct impact lead to a better understanding of the effect. Whether or not sub-terminal effects of toxicants at an individual level are ecologically relevant needs investigation. Ecotoxicological modelling, in particular individual-based population modelling, is a rising research area trying to overcome the mismatch between laboratory testing on individuals and the aim of environmental risk assessment to protect populations. Data as shown here not only contribute to the understanding of effects, but they can also be used for development and evaluation of models.

ET15A-5  
New methods for assessing the effects of insecticides on insect larvae and adult emergence in freshwater outdoor microcosms
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1Huntingdon Life Sciences, Eye, United Kingdom  
2Dow AgroSciences, Abingdon, oxfordshire, United Kingdom  
3Institute of Terrestrial Ecology, Silwood Park, Ascot, United Kingdom  
4European Agrochemicals Association (EA4C), Brussels, Belgium  
5Instituto de Investigaciones Agropecuarias (INIA), Madrid, Spain  
6Agreco Ltd, Jeju Island, South Korea  
7Envirosafe Solutions, Inc., Frederick, Maryland, USA  
8Lyons Consulting, Troutdale, OR, USA

In the classic test designs that follow current recommendations for freshwater microcosm studies, (HARAP, CLASSIC, and OECD), the effects of insecticides on the emergence of insects from aquatic systems are generally based on counts of their numbers in emergence traps that occupy a relatively small proportion of the water surface. In order to more accurately evaluate the sub-lethal effects of insecticidal effects on emergence, we developed a novel method based on the application of an experimental design where the effects of insecticides are evaluated within a microcosm by collecting and measuring the total numbers of insects emerging from a microcosm at different times after exposure.

The results of this study on microcosms with differing predator densities were described in this presentation. In the first, over 150,000 insects from 13 families were collected. Emergence times for some species e.g. Damselies (Coenagrionidae) and Mayflies (Baetidae) were clearly defined whereas others e.g. Caddis (Lynnephilidae) and the Phantom midges (Chironomidae) were protracted and spanned almost the entire experimental period. Insect numbers were sufficient for multivariate and univariate analysis but differences in emergence periods highlighted the need for a knowledge of life history to allow correct data interpretation. In the second study, where emerged insects were allowed to survive within the enclosure, larvae counts in invertebrate colonisers placed into microcosms showed that at least three insect taxa i.e. Lymnephilidae (Caddis), Coenagrionidae (Damsel) and Lestidae (Damsel), reproduced and deposited eggs back into the microcosms from which they themselves emerged.

The methods described here can be used to assess the effects of plant protection products on insect emergence and reproduction under realistic semi-field conditions which satisfies a key requirement for the determination of the environmental safety of insecticides. The insects identified in these studies that were capable of emerging and reproducing in the aerial microcosms formed by the field enclosures, provide a valuable resource for extending field methods in ecotoxicology.

ET15A-6  
Low concentrations of imidacloprid cause mortality of Gammarus pulex by interfering with feeding behavior
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2EAWAG, Dübendorf, Switzerland

Neonicotinoids are insecticides which interfere with invertebrate behavior, e.g. feeding, by mimicking neurotransmitter acetylcholine and stimulating the nervous system. If feeding behavior is impaired, neonicotinoids might cause mortality even in concentrations far below acute toxicity levels. We studied effects of the neonicotinoid imidacloprid on the aquatic invertebrate Gammarus pulex. G.pulex is an European amphipod decomposing organic material in streams. It is highly affected by imidacloprid: acute toxicity \( G.\text{pulex} \) (LC50 = 270 µg/L) is almost 800 times below the concentrations for fish.

We carried out two experiments (14d and 21d long), where we measured survival, feeding rate and lipid content. Both included two treatments (A, B) with two 1-d exposure pulses and one treatment with constant exposure to the corresponding time-weighted average concentration (C: 15 µg/L). Treatments A and B differed in their times between the two 1-d exposure pulses (short, long). In the 1st experiment, we observed only survival while during the 2nd experiment, we also observed feeding rate by weighing the food before providing it to G. pulex and after removal from experimental beakers. Lipid content was measured from organisms sampled at the end of the 2nd experiment, by the gravimetric method. For food we provided horse chestnut leaves, inoculated with the fungi Cladosporium herbarum. At the end of the 1st experiment, survival in treatment A was 70%, in treatment B 63%, in treatment C (TWA) 46% and in controls 82%. The difference in survival curves between treatment C and controls was highly significant (one-way ANOVA: \( p = 0.001 \)) and also the pulse treatments differed from controls (Repeated Measures ANOVA: \( p > 0.05 \)) while between
ET15B-1

Using ‘species sensitivity distribution’ to assess herbicides toxicity on benthic diatoms assemblages
F.A. Larras, A. Bouchez, B. Montuelle
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Herbicides in the littoral zone of lakes are often present in higher concentrations with greater molecule diversity than in pelagic zone. Benthic diatoms comprise a significant portion of aquatic primary producers in this zone, but the herbicides tolerance threshold of these non targeted organisms is not well known. With the view of assessing the ecological risk of herbicides for aquatic microorganisms, some models, such as Species Sensitivity Distribution (SSD), have been developed on phytoplanktonic species of the pelagic zone. Often, benthic communities are not considered in lake from an ecotoxicological point of view. However, these zones could be exposed to specific contamination and studying the sensitivity of benthic organisms could provide a new approach for ecotoxicity assessment in lacustrine environments. Our study aims to determine if SSD models are adapted to describe the sensitivity of benthic diatoms to herbicides. To achieve this goal, we assessed the sensitivity of 11 benthic diatoms species to 5 herbicides (diuron, isoproturon, terbutryne, atrazine and metolachlor). First, we constructed a database of toxicity thresholds for each herbicide-species combination using 96 hours monospecific bioassays and an evaluation of growth inhibition. From each dose-response curve, an effective concentration that inhibits 50% of growth (EC50) was extrapolated. EC50 values showed a great variability of sensitivity among species for a same herbicide and among herbicides for the same species. A SSD curve (SSD-EC50) was built for each herbicide, based on EC50 values of each species exposed to that herbicide. According to Hazardous Concentrations (HC) obtained from SSD-EC50 curves, the increasing order of herbicide toxicity was metolachlor, atrazine, isoproturon, terbutryn and diuron. Different pools of diatoms were defined according to their tolerance level. The most obvious pools distinction was associated with photosysten II (PSII) inhibitors. Four strains (Craticula accomoda, Gomphonema parvulum, Eoloma minus) of the 11 tested species were always the most resistant. This tolerance could be explained by the trophic mode and the motile guild. Indeed, N-heterotroph and motile guild species seemed to be more resistant to PSII inhibitors than N-autootroph and other profile guild species. These initial results are encouraging and support the use of SSD models for more complex investigations based on benthic diatom data.
ET15-6
Consequences of the Three Gorges Dam in China - Conceptual approaches to study the fate, bioaccumulation and effects of organic micropollutants in aquatic food webs and sediments of the Yangtze
B. Scholz-Starke1, Z. Chen1, T. Floehr1, H. Hollert1, J. Huo1, B. Li1, R. Ottermanns1, M. Roji-Nickoll1, B. Schmidt1, K. Strauch1, L. Wu1, D. Yin2, Y. Yuan3, X. Yuan4, A. Zoschke1, A. Schäffer1
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ET15 - Quantitative Structure Activity Relationship (QSARs) and similar models for predicting the toxicity of chemicals, mixtures and combined stress

ET16-1
VEGA, a new platform combining QSAR and read across for the prediction of chemical properties
E. Benfenati1, A. Manganaro1, R. Gorina Diaza1, A. Lombardo1, G. Gini2, M. Floris2, S. Moro2, F. Lemke1, T. Martin1, D. Young2
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ET16-2
Intracellular localization of chemicals
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Intracellular localization can to a large part be predicted from common physico-chemical properties, such as log Kow, pKa, valency and molar mass. The selective distribution of molecules in intracellular compartments such as membranes, mitochondria and lysosomes will also lead to specific diseases. Consequently, intracellular distribution may be a determinant of specific toxic effects of molecules. The cell model was used to predict the chemical space of compounds that local in cellular subcompartments, such as cytosol, acidic vesicles (vacuoles or lysosomes), nucleus, mitochondria and oleosomes. Accordingly, weak acids localize in cytosol and stronger in mitochondria. Weak mono- and bivalent bases accumulate in acidic vesicles such as lysosomes and vacuoles. Strong bases (i.e., cations) and weak bases that form cations with delocalized charge are attracted by the electrical potential of mitochondria. Lipophilic molecules (log Kow > 5) partition of course primarily into membranes and lipids. Similar predictions are obtained using the Horobin QSAR model. A validation study for mitochondrotropes gave about 2/3rd agreement. Disagreement could be explained in most cases. The intracellular localization is not always selectively, making literature references sometimes ambiguous. A recent experimental study gave good agreement of cell model predictions with the experimentally determined dynamics of uptake. In conclusion, the prediction of intracellular localization from molecule properties by QSAR and pharmacodynamic models is feasible and eventually may be exploited to identify new, specific modes of action.

ET16-3
Mitochondrotropics: A review of their mode of action, and their applications for drug and DNA delivery to mammalian mitochondria.
J Conr Release 121:125-136
Developing read-across justifications in REACH

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Under REACH, ‘alternative methods’ such as Read-Across and Grouping/Category are key tools for preventing unnecessary animal testing and reducing costs for dossier preparation. However, relatively little guidance on the identification of valid analogues and categories and whether categories will support only ‘interpolation’ or also ‘extrapolation’ is available. We will present and develop practical examples of what we think will be valid generalisation for these approaches. Read-across Read-across implies that measured data for one substance are used as surrogate data for another closely related substance, under the assumption that the chemical similarity of the two substances is high and therefore the biological similarity is also high. Thus, a single endpoint to an entire dataset, and may involve using available source end point data to substitute for unavailable target data or to argue that tests need only be done for the designated source. A testing proposal submitted by a client for 2 closely related alkylphenols argued that the test was only needed for one substance, with subsequent read-across to the other; prompted by a rejection from ECHA, we have developed more thorough justifications for read-across based on available toxicological data and an in-depth description of the chemistry of both substances. Category Category approach implies that classes of closely related chemicals (usually homologous series) exist for which it is possible to qualitatively interpolate properties for ‘missing’ substances (analogously to the quantitative argument for classical QSARs). For a long chain alcohol, we prepared an argumentation that category approaches may also be used for near-end extrapolation and category extension. ‘Mixtures’ Data availability is often a problem for UVCB substances; moreover, their variable nature presents significant challenges for testing and interpretation of results. Treating complex substances as mixtures allows the use of available data for individual constituents, if these are available, but also for ‘mixtures’ of the prescribed methods often lead to over-prediction of hazard and classification. ENVIRO used a mixture approach to develop an integrated data set for a well-defined reaction product. By careful evaluation of the data set, supported by limited testing and refining evidence for degradation and biocentronisation, we were able to justify more rigorous PNECs and avoid classification of the substance.

ET1-6

Immobilised artificial membrane (IAM) chromatography : an investigation of the effect of pH on ionisable species

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The octanol/water partition coefficient (log P) is used in many areas of ecotoxicology and modelling as a measure of hydrophobicity or lipophilicity. However it is well known that for ionisable chemicals or surfactants the measurement of log P is difficult due to the tendency of these types of materials to sit on the octanol/water barrier. Similarly, the accuracy of the prediction of log P for these types of materials is variable depending on the model used to calculate it. However, even accurate determination of log P does not always lead to accurate in silico correlation with toxicological endpoints. In part this is due to differences between octanol/water partitioning and partitioning across biological membranes. Phosphatidylcholine (PC) is a ubiquitous phospholipid which is a major component of biological membranes. Immobilised Artificial Membrane (IAM) Chromatography utilises a phospholipid membrane made of PC. Using PC as a stationary phase and aqueous or organically modified buffers as a mobile phase, a partition coefficient (kIAM) for chemicals can be determined as a measure of the ability of a chemical to partition into the liposome (phospholipophilicity). This technique has been shown to have good correlation with toxicological endpoints for neutral compounds where log kIAM is measured. As in biological systems and in buffers, ionisable compounds exist in different ionic states which means that they interact to a different extent with biological membranes. The methods used to determine log P in these systems do not sufficiently allow for this which is why in part that correlation of ionisable chemicals with toxicological endpoints becomes reduced. This study was performed to investigate the effect of the pH on the partitioning of the chemicals in their ionic and neutral forms using IAM chromatography. As expected, the data indicated that the retention of ionisable chemicals is affected by the pH. Chemicals had a value for the neutral form (log kIAM) and the ionised form,(log kIAM (ion)) under relevant extreme pH conditions. However, at pH between these extremes, retention of the chemicals were based on an aggregate of both neutral and ionised species. It was also recorded that this aggregate retention indicated that the extent of ionisation of the system was not directly linked to the pKa of the compound under aqueous conditions but was shifted positively along the pH scale for acids and negatively for bases.

ET1-5

Simulation of metabolism and modelling environmental fate and (eco)toxicity of chemicals


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Information regarding the metabolism of xenobiotic chemicals plays a central role in regulatory risk assessments. Because so many new chemicals are being produced than can be available in an early stage of testing and assessment priorities undergo thousands of untented chemicals requires methods for predictive hazard identification. Metabolites and breakdown products may occur in every environmental compartment, in animal and human food causing unexpected changes of evolutionary established biological communities or chronic adverse effects. For this reason the data on extent of formation of metabolites is required by regulatory authorities as specified in technical guidance documents. The aim of this presentation is to share our experience in the computerized management of metabolic data and development of simulators of metabolism for predicting environmental fate and (eco)toxicity of chemicals. Mathematical formalism and its adaptation for modeling environmental fate (abiotic and microbial degradation, bioaccumulation) and (eco)toxicity of chemicals (acute aquatic toxicity, skin sensitization, genetic toxicity) will be presented. Model performance, applicability domain and interpretation of model predictions and supplementary information will be also discussed.

ET1-6

Integrated screening classifier for substance-specific waiving of biodegradation assays in PBT assessments

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The implementation of the REACH regulatory framework requires the assessment of persistent, bioaccumulative and toxic (PBT) potential for substances that are produced or imported in amounts of 10 or more tons per year. The PBT assessment entails detailed substance-specific testing, which involves substantial use of animals and significant economic costs. In this context, the use of intelligent testing strategies (ITS) results in a cost-effective assessment scheme. A key element for the success of the ITS is the use of non-testing methods as complementary sources of evidence to waive specific bioassays. Therefore, the use of ITS to optimize the costs of regulatory risk assessment requires the development of highly reliable non-testing methods suitable for test waiving. Persistence is a fundamental property that strongly determines the potential environmental impact of a chemical. Consequently, the objective of the current work was to develop and validate an integrated screening classifier specifically designed to identify non-ready biodegradable chemicals with high reliability (i.e. low risk of false negatives). Results obtained demonstrated that the use of ensembles of weak classifiers, tuned to distinguish between ready and non-ready biodegradable chemicals, constitute an appropriate approach to develop screening models suitable to identify non-ready biodegradable chemicals with high accuracy (99.7% and 100% respectively). The low false negative rate of the above ensemble classifiers allows its integration within the decision-making process of an ITS to waive biodegradation testing for certain chemicals. The bioassay waived may vary from the role of PBT assessments in terms animal use and economic resources needed, thus providing a cost-effective implementation of the REACH regulations.

ET18 - Tropical ecotoxicology

ET1-8

Assessing the toxicity of herbicides mixtures used in sugarcane culture in Brazil using zebrafish

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Brazil is a pioneer in large-scale use of ethanol, a renewable fuel that contributes to the reduction of greenhouse gas emissions by combustion of fossil fuels. The high production of sugarcane crops demands the intensive use of water and inputs that can compromise water bodies adjacent. Herbicides are the most common pesticide used in this crop, but the impact of this practice on non-target organisms is not well known. Moreover, possible interactions between different herbicides used simultaneously are not understood. Most studies into herbicides ecotoxicology are related to temperate ecosystems thus, there are still many gaps about its effects in a tropical climate. The aim of this work was to evaluate the effects of the mixture of ametryn (AMT) and diuron (DIU), herbicides widely used in sugarcane crop, to the tropical zebrafish (Danio rerio). Binary combinations of DIU and AMT were tested as follows; 0, 4.8, 6.8, 9.6, 13.6, 19.2 mg L-1 of DIU and 0x (soil control) and, 0, 5.7, 13.7, 19.4, 27.4 and 38.8 mg L-1 of AMT. The trials were based on the OECD protocol on Fish Embryo Toxicity Test. Several sub-lethal and lethal endpoints were evaluated. The activity of the biomarkers CHE, LDH and GST was determined after 96 h of embryo exposure to the binary mixture; 0x, 2.5, 5.0, 7.1 and 10.01 mgL-1 DIU and 0x, 0, 5.1, 7.2, 10 mgL-1 of AMT. Sigma Plot 10.0 package was used for statistical analyses and ToxCalc spreadsheet were used to calculate LC50-96h and determine the model that best describes the mixture. LC50-96h values for zebrafish exposed to AMT and DIU were, respectively, 48.46 (2.2) mgL-1 and 23.93 (1.24) mgL-1. The model which best describes the mixture was the inhibition dose response model with toxicity dose level dependent: synergism in low and antagonism in high dose. GST activity was induced by AMT. However, the mixture had higher concentrations of DIU we observed a reduction in activity. We observed an inhibition of CHE in higher concentrations of the two components of the mixture and no influence on LDH activity. We determined that the model which best describes biomarkers activity for these enzymes are concentration
addition. Our results show that there is a synergism between aminopyralid and diuron, which enhances its effects on Danio rerio. Risk assessment which uses data from the single applications can be established of the pesticides, and outputs were obtained for the two scenarios to be compared, i.e. tropical and non-tropical conditions. Results from modelling show that except for a

ET1-8

Baseline levels and trophic transfer of persistent organic pollutants in sediments and biota from the Congo River Basin (Democratic Republic of Congo)

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The present study aims to evaluate the occurrence of persistent organic pollutants (POPs) in the Congo River Basin (CRB) and to investigate the trophic transfer of POPs through a tropical freshwater aquatic food web using stable isotopes. To our knowledge, no data on levels of POPs in sediment and biota from the CRB are present in the literature. In addition, studies on biomagnification profiles of POPs are scarce in tropical regions. Samples of sediment and biota (i.e. invertebrate and fish species) were collected from three tributaries (Lomami, Aruwimi and Itimbiri) and the Congo River itself to determine the degree of contamination by POPs (PCBs, PBDEs, DDTs, HCHs, CHLs and HCB) with a GC-MS.

Stable isotope (δ13C and δ15N) measurements in plant, invertebrate and fish tissues showed that the degree of contamination by POPs (PCBs, PBDEs, DDTs, HCHs, CHLs and HCB) is comparable to results of other studies from temperate and tropical regions, with exception of total PCB levels found in fish from the Itimbiri River (average 2.3PCB = 146±920 ngg lipid weight lw). The PCB levels are of the same magnitude as found in industrialised basins. This might be related to the presence of industrial and agricultural (palm oil) activity in the Itimbiri River Basin. Of the POPs analysed, total PCBs were the predominant contaminants with concentrations ranging from 21 to 3664 ngg lw, followed by DDT and its metabolites at 4 to 504 ngg lw, PBDEs at 2 to 188 ngg lw and HCHs at 2 to 66 ngg lw. The values of 813C and 815N in biota ranged from −20.5 to −22.6 and from 2.3 to 16.2, respectively. On average, 815N increased from primary producer to invertebrates to fish. The understanding of the trophic transfer of environmental contaminants in biota of the CRB is critical to evaluate the influence of these contaminants on ecosystems and human life. The observed increase in 815N with increasing PCB 153 concentrations indicates that trophic levels play an important role in the movement of contaminants through the Itimbiri food web (R2= 0.66, p<0.0012). The slope of the regression equation (0.35) is an index for biomagnification. These correlations can be established for different individuals. Slopes will be compared with results from other studies around the world and will be used for risk assessment.
High temperatures in the tropics also foster volatilisation rates, while high precipitation and poor soils tend to increase losses into runoff and, for certain chemicals, their organic contamination in soil. To study the impact of these parameters on the dissipation and sequestration of veterinary antibiotics, we conducted laboratory experiments in suitable microcosms. In laboratory experiments, the dissipation of SDZ proved to be accelerated by a factor of two to three with a temperature increase of 11°C. We successfully used the temperature-dependence of dissipation rate constants that was derived from these laboratory experiments to predict temporal changes of SDZ concentrations in the field experiment. When the soil moisture was either very high or low, however, systematic deviations between measured and predicted concentrations were observed, particularly for the soil incubated as slurry. In the long-term experiments, the radioactive balance revealed that 99% of the applied radioactivity was still detectable in soil. Revealed only a minor impact of the rhizosphere on the fate of the drug applied. The mineralization rate (<0.5%) and the plant uptake (<0.5%) were negligible over the whole investigated period. The radioactive balance revealed that 99% of the applied radioactivity was still detectable in soil. Furthermore, the results revealed that up to 70% of the applied compounds in the root area were degraded to unknown metabolites. In order to correlate chemical distances from the roots of the plant revealed the considerable impact of this biological hotspot. The mineralization rate near the roots was 10 times higher than for bulk soil. The results revealed that up to 70% of the applied compounds in the root area were degraded to unknown metabolites. In order to correlate chemical and biological data, we performed an analysis of the abundance of bacterial resistance genes in the rhizosphere. The results revealed only a minor impact of the rhizosphere on the fate of the drug applied. The mineralization rate (<0.5%) and the plant uptake (<0.5%) were negligible over the whole experimental period. The radioactive balance revealed that 99% of the applied radioactivity was still detectable in soil.

ET19A-3 Fate of sulfadiazine and its effects on abundance and transferability of antibiotic resistance in rhizosphere and bulk soil
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Veterinary antibiotics reaching agricultural lands with manure pose the risk of spreading of antibiotic resistance. The rhizosphere is a hot spot for horizontal gene transfer and might facilitate the degradation of antibiotics. On the other hand, it was shown that the addition of artificial root exudates increased the bacterial community tolerance towards the antibiotic compound sulfadiazine (SDZ), indicating that the rhizosphere might be a hotspot of resistant bacteria. On the other hand, the exposure of bacteria to SDZ is presumably reduced in the rhizosphere since the dissipation of bioaccessible SDZ-concentrations was recently shown to be accelerated in rhizosphere soil. However, little is known about the abundance and dynamics of sulfonamide resistance genes in the rhizosphere. We therefore compared the fate and effect of SDZ in bulk- and rhizosphere soil. This study focuses on the abundance of resistant bacteria on the one hand and the exposure of bacteria to SDZ on the other hand. The results revealed that the development of a new analytical method to detect plant uptake of the applied drugs yielded results revealing just trace amounts (maximum of 1% of the applied compounds) mainly in the roots (99%) of the maize plants. The experimental setup using the fluorquinolone DIF revealed only a minor impact of the rhizosphere on the fate of the drug applied. The mineralization rate (<0.5%) and the plant uptake (<0.5%) were negligible over the whole experimental period. The radioactive balance revealed that 99% of the applied radioactivity was still detectable in soil.

ET19A-4 Degradation of sulfadiazine in lysimeters, soil microcosms, and pure cultures
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Among sulphonamides, sulfadiazine (SDZ) is one of the most widely applied veterinary antibiotics. Long-term lysimeter studies with manure from pigs treated with 14C-labeled sulfadiazine (SDZ) gave the first hint that considerable amounts of the radiolabelled SDZ applied to the soil could have been mineralized within 3 years, although, up to now, mineralization of SDZ in soils was supposed to play only a minor role. Three years later the measurement of the metabolite patterns in the phase and in rhizosphere pore water without further sample clean up and at high spatial and temporal resolution. In addition the usage of 14C-labelled compounds will reveal the mass balance of the applied drug including formation of NER (non-extractable residues) and mineralization.

The influence of the mass balance of the fate and behaviour of two veterinary antibiotics sulfadiazine (SDZ) and diltiazem (DIF) and their metabolites in soil was investigated in microcosms. Radioactivity was traced in water and I C-SMS analyses of pore water was performed. The pore water analysis of soil at various distances from the rhizosphere of maize plants revealed no influence on the fate of the applied compounds. After incubation for 55 days with SDZ the soil analysis at various distances from the roots of the plant revealed the considerable impact of this biological hotspot. The mineralization rate near the roots was 10 times higher than for bulk soil. Furthermore, the results revealed that up to 70% of the applied compounds in the root area were degraded to unknown metabolites. In order to correlate chemical and biological data, we performed an analysis of the abundance of bacterial resistance genes in the rhizosphere. The results revealed only a minor impact of the rhizosphere on the fate of the drug applied. The mineralization rate (<0.5%) and the plant uptake (<0.5%) were negligible over the whole experimental period. The radioactive balance revealed that 99% of the applied radioactivity was still detectable in soil.
Effects of sulfadiazine-contaminated pig manure on abundance, diversity and activity of microbes involved in nitrogen transformation in different soil compartments after repeated application of the antibiotic sulfadiazine.

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A kinetic model that couples environmental fate processes of antibiotics with an effect model of bacteria inhibition was studied. The model uses the antibiotics concentrations in soil as input. It requires a number of input parameters which are more or less uncertain. A probabilistic Monte-Carlo type methodology was used to estimate the uncertainty of selected output variables depending on uncertainty estimates of input parameters. Additionally, the contribution of single parameters to the simulated variability of the output is analyzed and expressed by the contribution to variance. The uncertainty of the output variables is characterized by the 10th and 90th percentile of the frequency distributions. As an example, sulfadiazine (SDZ) effect on the density of ammonium-oxidizing bacteria (AOB) in soil was investigated. After manure application to soil, simulated SDZ concentration decreases monotonically with moderate uncertainty in the first 25 days. The time point at which the available SDZ concentration falls below the inhibition constant of AOB (0.025 mg/kg) is predicted to be most likely between 22 days (10th percentile) and 33 days (90th percentile). Increasing AOB densities over the first 15 to 50 days are predicted due to enhanced growth after amendment of ammonium containing manure. Once all ammonium has been consumed, AOB density decreases again. Uncertainty of the input parameters manifests itself in a difference of approximately factor 1.6 between the 10th and 90th percentile of AOB densities. A delaying effect of SDZ on AOB growth is predicted by the coupled model. In the first 22 - 33 days available SDZ is above the inhibition constant, growth of AOB is suppressed, but starts after SDZ has dissipated. The uncertainty is larger in the coupled model because it additionally carries the uncertainty of the chemical fate parameters. The contribution of the chemical fate parameters on AOB uncertainty is small compared to the parameters of the biological model. Only the hydrolysis rate constant contributes to more than 20%. The analysis shows that the combined parameter uncertainty does not compromise the conclusion of the delaying effect of SDZ on AOB growth after manure application. The probabilistic simulation shows that the chemical fate model, the AOB growth model and the coupled model deliver interpretable predictions of the dynamics of the important output variables SDZ and AOB, even with the uncertainty of the input parameters.
SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting

In vitro effects of 17β-trenbolone on the mRNA levels of steroid hormone receptors, Growth Hormone and gonadotropins in pithitary glands from rainbow trout (Oncorhynchus mykiss)
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Based on the high biological activity of trenbolone in fish and on the doses used by builders, the compound might potentially pose a risk to the aquatic environment also in EU although not officially present. Previous studies in our laboratories have shown that trenbolone is capable of irreversibly changing sex ratios to 100% males in zebrafish at water concentrations in the low ng/l level. The present study was undertaken to investigate effects of trenbolone on mRNA levels of steroid hormone receptors, GH, LH and FSH in pithitary glands from rainbow trout after in vitro incubations of the excised tissues with the synthetic androgen.

ICP-1 and AR mRNA levels in liver slices incubated with trenbolone at concentrations from 5-100 ng/ml were unchanged

Pithitary glands were incubated with trenbolone from 1.35-1355 pg/ml:
- No significant changes were found in GH mRNA levels
- AR mRNA was significantly reduced at all concentrations
- FSH mRNA was significantly increased at the highest concentration
- LH mRNA was massively and inversely reduced with incubation concentrations

LC01 - Development in life cycle inventory analysis and modelling

LC01A-1 Market-based allocation of recycling benefits
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Market-based allocation of recycling benefits has already been described since the years 2000. However, this approach tends not to be largely adopted in international standards.

In practice, there are mainly three types of benefit allocation with system expansion modeling: (i) allocation of benefits to the producer bringing material on the market (that can quite easily be identified at the end-of-life); (ii) allocation to the product incorporating secondary material and (iii) 50/50 allocation (half of the benefits of recycling at end-of-life and half of the benefits of incorporating secondary material are accounted for).

Market-based allocation is dictated by the answers to the following questions: “Which additional amount of secondary material will be exchanged on the market if supply increases thanks to the appearance of a new source of secondary material?” or “Which additional amount of secondary material will be exchanged on the market if demand increases thanks to the appearance of a new producer of a good based on the secondary material?” Analysis of a price elasticity of demand and supply provides answers. This paper aims at depicting several typical supply-demand curves and at associating these to actual market situations (for example, what if there is a back obligation or subsidized waste collection, etc.). The appropriate market-based allocation can hence be dictated in each case.

Namely, allocation to the supplier (i) to be promoted in cases where the supply is fully inelastic and where the demand is fully elastic. Examples of such markets are, among others, PET and packaging gas.

In markets where supplies to answer and demand variations are more equilibrated, a 50/50 allocation should be used, as it is the case for certain types of paper and boards.

Market situations for main materials are analyzed so as to provide sound justification for standard developments.

LC01A-2 Using a long-term energy model for the consequential and prospective life cycle assessment of the use of biomass based synthetic diesel (BTL) in France
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Consequential Life Cycle Assessment (C-LCA) has been developed recently as a modeling approach which captures environmental impacts of a product beyond direct physical relationships accounted for in Attributional LCA. This approach seems interesting for the evaluation of biofuels since their indirect impacts on other sectors (agriculture, forestry, electricity production) may be important. In this study, we use a prospective optimization model representing the French energy production and transportation sectors (agriculture is partially included due to the presence of biofuels) to evaluate the impacts of the production of BTL in France in the time horizon 2007-2030. The model was developed with the economic model generator TIMES.

Part of the work consisted in adapting the model to perform C-LCA integrating energy consumptions and emissions factors to the technologies described in the model. Scenarios were built in order to expose the C-LCA methodological issues we aim to discuss. We observed how the model behaves when applying variations to: the technology used for BTL production, the levels of development of BTL in France and the energy policy. Global Warming Potential (GWP) was calculated for each scenario using time-dependent characterization factors for greenhouse gases. Preliminary results show that the long-term energy model can be applied for measuring the indirect effects of the development of BTL in France. For example, it was possible to identify the source of the electricity that is going to compensate the supplementary demand for electricity due to the production of BTL using an allthermic process. This type of model presents some advantages in relation to other models previously used in C-LCA:
- It allows a fine description of transformation steps of primary resources in energy carriers.
- It allows the observation of marginal and non-marginal perturbations on the energy sector.
- Emissions are exogenous to the model. Most of the other C-LCA studies use economic equilibrium models to quantify the consumption of certain products and the emissions are integrated manually afterwards.

Nevertheless, in this version of the model, the agricultural sector is only described partially and land use changes (direct and indirect) can’t be evaluated. A whole world description of the agriculture and forestry sector would be necessary for this type of analysis. One way of improving this C-LCA would be to integrate our model with a general equilibrium model.

LC01A-3 Modelling land use changes in consequential LCI: limitations of equilibrium models
- Public Research Centre Henri Tudor, Luxembourg: kirchberg, Luxembourg
- Ordecsys, Chêne bourg, Switzerland

This presentation aims at discussing the use and limitations of economic equilibrium models for the development of consequential LCI, with a specific focus on the case of maize production from maize in Luxembourg. The core research objective is the development and application of a Partial Equilibrium (PE) model representing the market constraints and reaction to the demanded production of maize and providing: 1) the change of crop production patterns, i.e. the primary consequences on Luxembourg’s agriculture system. Forage crops are included, making the link to the consequences on meat and milk production. 2) The changes of land use type and related primary consequences in terms of modified pollutant emissions and land transformation impacts. The modelled changes are then propagated inside the national economy, using a general equilibrium model (more specifically the equilibrium model used in the case of Luxembourg and outside the national boundaries to account for additional environmental impacts. So far the results show no need for intensification of the existing and new areas to meet demand for maize. To study the impacts of increased agriculture prices, we increased the import prices of the “agriculture” commodity by 10% in Luxem calibrated to the base year 2005. We find that the share of expenditure by households on agriculture production increased by 2%. Agricultural land, in the value added in the economy and increased demand for maize which may displace existing crops will not have serious economic impacts. These findings were corroborated using the global model GTAP, which was used to evaluate the increased demand for displaced agriculture crops on account of additional production of maize. However, since the PE model is based on revenue maximization, it is difficult to properly consider non-economic constraints such as behaviours related to habits, cultural heritages or additional regulatory constraints. Also the modelling of the influences of crop production patterns on the food sectors is not trivial because of the difficulty of assigning a clear and robust aggregated market relation between forage crops and meat and milk.
Equilibrium models fall short in including non-technical and non economical constrains. The complementary use of agent-based modelling could be an alternative approach for proper consequential LCI.

LCOA-4

Integrating accident-related methods and impacts into the life cycle toolbox

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Life cycle assessment is a tool and more understood to be ‘only’ one component of a wider environmental and even sustainability assessment toolbox. Enhancing classical Life Cycle Inventory (LCI) modelling by integrating accidents in production, use and end-of-life of products is one important step towards more complete evaluation of product systems’ life cycles. LCA according to ISO 14040 deals implicitly with non-accidental (‘regular’) releases only, while some accidental releases may be included due to how LCA data is collected (e.g. site’s yearly average, including leakages etc.). There are however important organisational and decision-making related reasons for managing these two types of generation separately. Accident prevention in industry is done separately from dealing with normal releases to the environment. A separate inventory of accident related releases and casualties (Life Cycle Accident Inventory, LCAI) is hence required for effective decision support. Several studies present case-specific solutions for this issue and some general method concepts have been presented. A systematic and consistent methodological solution for full implementation is still lacking. Joining LCA methodology and tools from Risk Assessment (Event Tree Analysis and Fault Tree Analysis) yields a powerful approach for integrated analysis of the environmental and health effects from accident scenarios in industry.

This paper presents a novel approach assessing indirect impacts from water use due to compensation scenarios. In this work, a more systemic approach is advocated. Relying on energy systems analysis results, the purpose of the study is to analyse to what extent considering substitutional effects and back-up can help characterise power generation from intermittent resources. The study starts with describing the German electricity grid in 2006 with and without wind power generation. By using the results from an agent-based energy system model, the issues of intermittency and the backup needed for wind power were addressed. Through substitution of fossil fired capacities, the assessment considering these consequences led to an even better environmental performance of wind power in all of the considered impact categories (including global warming) compared to the LCA not considering these consequences, more particularly for mineral resource depletion and natural land transformation. As this study could not fully resolve the issue of finding a functional unit that is more appropriate than the kWh for comparing different power generation technologies, other approaches are also discussed.

LCOB-1

Book, trade and claims in LCA: how to model certificates delinked from physical flows

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In May 2011, the association of issuing bodies AIB announced that 1 billion EECS certificates (which equals 1 billion MWh of electricity) have been issued in Europe since its start nine years ago. There is a large demand in recent energy certificates, which helps electric utilities and companies from the manufacturing as well as service sector to reduce the environmental impacts of the electricity they purchase.

The international standards on life cycle assessment (ISO 14040; ISO 14044) do not specify how certificates should be taken into account when performing a product or company LCA. This methodological LCA study demonstrates that recently established carbon footprint standard are clear with regard to carbon offsetting measures (emission certificates): these are considered as an improvement measure and shall not included in the product LCA but kept separate.

Up to now, the role of renewable energy certificates has not been discussed widely. This presentation shows the mechanism and volume of RECS certificates as well as its consequences considering these consequences led to an even better environmental performance of wind power in all of the considered impact categories (including global warming) compared to the LCA not considering these consequences, more particularly for mineral resource depletion and natural land transformation. As this study could not fully resolve the issue of finding a functional unit that is more appropriate than the kWh for comparing different power generation technologies, other approaches are also discussed.

LCOB-2

Using water markets and consequential LCA to assess indirect impacts from water use

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Using water can incur direct impacts on human health from water deprivation for domestic use or agriculture. However, these impacts do not occur in regions where economic resources are sufficient to allow the deprived users to turn towards technology to meet their needs. On the other hand, this technology leads to burden shifting that should be captured in a comprehensive assessment of water use in LCA. This paper proposes a consequential framework and model using water markets and marginal technology in order to assess LCA indirect impacts from water consumption and degradation, by identifying marginal processes and including their life cycle inventory. The model is based on an agent-based energy system model, the issues of intermittency and the backup needed for wind power were addressed. Though substitution of fossil fired capacities, the assessment considering these consequences led to an even better environmental performance of wind power in all of the considered impact categories (including global warming) compared to the LCA not considering these consequences, more particularly for mineral resource depletion and natural land transformation. As this study could not fully resolve the issue of finding a functional unit that is more appropriate than the kWh for comparing different power generation technologies, other approaches are also discussed.

LCOB-3

Modelling of biogenic CO2 fluxes in LCA and their integration with the global C cycle

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In Life Cycle Assessment (LCA), the same characterization factors are conventionally applied irrespective of when the emissions occur (the same importance is given to emissions in the past, present and future). This accounting paradigm makes bioenergy systems climate neutral as long as they are carbon neutral (the same amount of CO2 released from combustion is sequestered by growing trees). When the analysis is constrained by specific time boundaries the validity of this convention becomes shaky. We stress the fact that the equivalency between C and climate neutrality in bioenergy LCA ignores the temporal gap between the emission (usually at a single point in time) and the greenhouse gas effect, which is usually distributed over many years. Neglecting these dynamics has an influence on the final climate impact assessment. The challenge for LCA practitioners is to model these fluxes and calculate the resulting climate impacts with unit-based indicators that be included in LCA case studies.

In this work, we elaborate on this issue using relatively simple mathematical methods which provide simulations of the carbon flow dynamics of biomass systems. Probability distribution functions are used to include in LCA the dynamic profiles of CO2 emissions and removals associated with biomass management for bioenergy and wooden products. Following an approach based on Impulse Response Functions, CO2 atmospheric profiles are calculated together with the respective changes in radiative forcing. Results show the importance of using emission and removal functions rather than single pulses or linear amortization procedures, which generally overestimate the climate impact of CO2 emissions, especially in presence of short time horizons and for relatively wide time-distributed emissions. This set of flexible and can provide guidelines for this approach to be seamlessly applied in LCA. Following this, we believe that a debate should be open in the LCA community to properly address timing of emissions and revisit the climate metrics used in the assessment. Besides normalized metrics as GWP, absolute metrics showing how the impacts change over time are preferable and can provide additional insights on the effective mitigation potentials of the system under study. The approach presented here deserves future research for a possible use in accounting of emissions from harvested wood products and in processing data by emission inventory experts within the Kyoto protocol and its successor.
At the same time, inequalities between and within societies remain high - leaving billions with unmet basic human needs and a disproportionate vulnerability to global change. Every day, unsustainable patterns of consumption and production methods as well as population growth challenge the resilience of the planet to support human activities. However, humans are not machineries and they are driven by flows of information, knowledge, educational and cultural experience, and so forth. These are essential items for our future sustainable development but how to integrate them into human labour LCI profiles remains an open task.

**LC01B-4**

**Evaluating the greenhouse gas emissions of retrofitting the existing Irish housing stock - A combination of process analysis and input-output analysis**

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Historically, in Ireland focus has been on the domestic greenhouse gas emissions to the neglect of the emissions attributable to imports in retrofitting the existing Irish housing stock. This paper reports on a life cycle study of energy efficient retrofit options for the existing Irish housing stock with a focus on the significance of greenhouse gas emissions due to imports in order to depict the true total emissions attributable to retrofitting the existing Irish housing stock. While typical commercial LCA software tools was used to assess environmental impacts due to imported goods, domestic generated emissions including those due to imported products induced by addition of energy inputs were evaluated using input-output analysis. Results show that imports caused about 50% of the total greenhouse gas emissions. Although operational phase consumption and emissions was much greater than any other phase, there was a wide variation in the impacts on this balance across the retrofit options. Using these results an appraisal is provided of the benefits of utilizing process-based hybrid LCA to provide greater information to aid policy making on the life cycle performance of existing dwellings.

**LC01B-5**

**A framework for prospective hybrid life-cycle assessment and its application to energy technologies**

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Life-cycle assessment and input-output analysis literature abounds with case studies that have been developed at a particular place and a particular time in the past, being as many photographic representations of specific past situations. In this literature, there is an absence of future-oriented work that attempts to predict future situations in which products will be produced, utilized and disposed are rare. Some are based upon optimization and equilibrium models. With the remarkable exception of the NEEDS project, future life-cycle inventory or input-output databases are not available. There is nonetheless an ever-increasing need for a life-cycle capable scenario modelling framework. We propose here a procedure to adapt an existing life assessment (LCA) databases and multi-regional economic input-output (MRIO) tables to future years (up to 2050) according to various external parameters that have been identified as central in determining the environmental impacts of the overall economy. The EXIOPOL project has recently produced a highly detailed input-output model; we utilize this model to build a life-cycle based framework for scenario modelling. A combination of existing prospective studies, including International Energy Agency's are used to produce a consistent hybrid model of the world economy which can serve as the background for prospective life-cycle assessment studies. We present the model development and provide some basic comparisons of the scenarios implemented. Finally, in order to exemplify the use of such a prospective hybrid LCA model, we apply it to the integrated environmental assessment of selected low-carbon electricity production technologies.

**LC02 - Developments in life cycle sustainability assessment**

**LC02A-1**

UNEP/SETAC approach for a life cycle sustainability assessment of products - just in time

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This publication describes life cycle techniques that can measure sustainability and allow LCA to support decision-making toward more sustainable product and process systems. An Environmental LCA looks at potential impacts to the environment as a result of the extraction of resources, transportation, production, recycling and use and discarding of products; life cycle costing (LCC) is used to assess the cost implications of this life cycle; and social life cycle assessment (S-LCA) examines their social consequences. However, in order to get the 'whole picture', it is vital to extend current life cycle thinking to encompass all three pillars of sustainability: (i) environmental, (ii) economic and (iii) social. This publication shows how all three techniques - which all share similar methodological frameworks and aims - can be combined to make the move towards an overarching LCSA possible.

Conclusion

LCSA has significant potential to be used by enterprises, governments, agencies for international cooperation and other organizations in society (such as consumers' associations) in their efforts to produce and consume more sustainable Still more research and applications are needed, but its application is already feasible and encouraged to speed the learning curve of the society.

**LC02A-2**

Challenges in implementing Life Cycle Sustainability Assessment (LCSA) and in an LCSA-based decision-making

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Life Cycle Sustainability Assessment (LCSA) has been proposed to be a combination of three assessments: environmental, economic and social (incl. socio-economic). In this way LCSA tries to make a bridge between the traditionally environmental oriented and generic life cycle assessment (LCA) and the more site- and time-specific sustainability assessment (SA), which takes into account all three generally accepted pillars of sustainability (economic, social and environmental). In the process of trying to bridge and to draw from the strong characteristics of SA and LCSA, LCSA encounters its own challenges.

**LC02A-3**

Sustainability and human labour: how can LCA answer?

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In LCA the intrinsic dependence of productions on human labour (HL) is usually disregarded, without providing any clear argument. Apparently, HL is not considered to be related to and affected by, changes to the functional unit. Nevertheless, HL could be seen as the common numeraire among the three pillars of sustainability: environment, economic and society. This research aims at investigating the relationship between HL and LCA and at developing an operational framework to include HL in LCA. We defined three HL types (qualified worker, technician, manual worker). A comparative LCA of the HL types was carried out using an environmentally extended input-output model of EU-27. Afterwards, ten agri-food and industrial LCA case studies were modified for hybrid LCAs, adding HL inputs to existing LCIs. The LCIA comparison showed that a qualified worker always generates impacts greater than other workers. The impact of average HL is higher in EU countries with the highest household expenditures’ level, which draws a clear connection with economic and (iii) social. This publication shows how all three techniques - which all share similar methodological frameworks and aims - can be combined to make the move towards an overarching LCSA possible.

Conclusion

LCSA has significant potential to be used by enterprises, governments, agencies for international cooperation and other organizations in society (such as consumers’ associations) in their efforts to produce and consume more sustainable Still more research and applications are needed, but its application is already feasible and encouraged to speed the learning curve of the society.

**LC02A-4**

What life cycle sustainability assessment does and does not for new and innovative technologies

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Performing a sustainability assessment of new and innovative technology is a complex task, as showed by the definition itself, which refers to two big issues: Technologies...
and Sustainability. Technologies can be classified in many ways, depending on the different typologies, development levels, effects and impacts on sectors, territories, markets, etc. The relation with sustainability is twofold, because technology can be considered both a cause of many environmental problems and a key to solve them. In fact, technologies are today considered the main agent of the present industrial, economic and social evolution and the main cause of the high speed of the present changes. As far as sustainable development is concerned, they are very controversial and disputed at scientific and social level. Indeed, the sustainable development cannot be considered simply a goal, rather a social process where shared sustainability principles are considered as starting point for assessing decisions through an interactive learning process.

Being sustainability a global concept, inevitably calls for a sides-wide analysis, a perspective that is at the core of the life cycle approach. A framework for life cycle sustainability assessment has been proposed, namely LCSA, which requires the application of LCA, Life Cycle Costing (LCC) and Social Life Cycle Assessment (S-LCA) under specific consistency requirements. The framework has been applied to the assessment of an innovative technology, in order to test its applicability, and it turned out to be a challenging task, mainly in relation to the following aspects: use of identical system boundaries; data availability and their significance; functional unit definition, especially in the case of S-LCA, and scenarios vs. product analysis. On the other side, the framework showed also its strengths in forcing practitioners in thinking about the different options and thus in detecting important aspects that at first sight could be considered negligible. However, we suggest supporting the application of the present LCSA with other methods and tools, able to take into account also aspects like the different ethical values, risk elements and the concept of social acceptance. Thus, LCSA can learn from the field of Technology Assessment in the way in which the problem is dealt with: the technology is at the core of the analysis, but the infrastructure and the organisation around it are equally important ingredients.

LC02A-5
Best Available System (BAS) for municipal solid waste management in developing Asian countries
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Fast growing mega cities in developing Asian countries are faced with municipal solid waste management (MSWM) related problems due to rapid urbanization, industrialization and population growth. Proper waste management with energy and material recovery prolongs the life of existing landfills, contributes in providing renewable energy and reduces pollution.

Although the need of shifting from landfilling to more environmentally sound solutions of solid waste management system is obvious, developing Asian countries have their own way of thinking, lifestyle, culture, budget related issues and concerns that reflects on their social and economic priorities in their decision making. For example, the fact that many scavengers live depends on collecting and reselling plastic waste from the landfill to private recycling plants may interfere with the calculus value requirement of an incineration plant.

This study attempts to take into account the social and economy related considerations in Life Cycle Assessment (LCA) by using an integrated, survey based methodology called Environmental Load Point (ELP) developed in Nagata laboratory of Waseda University. ELP has broadened indicators categorized in 9 impact categories, which are energy depletion, global warming, ozone layer depletion, acid precipitation, resource consumption, air pollution, ocean and water pollution, waste disposal, and ecosystem influence. The ELP results are weighted with weight factors from the national newspapers to find out the importance of each impact categories in the concerned country. Additionally, Life Cycle Cost (LCC) is combined with ELP results to give cost estimation of the constructed scenarios. This combination is called Best Available System (BAS) for MSWM. ELP represents the social considerations while LCC represents the economy factor of sustainability.

Different scenarios are constructed in this study with different portion of waste being recycled and incinerated, considering the composition of local waste and number of employment opportunity of scavenger in the new systems. The result is weighted with weight factors from the national newspapers to find out the importance of each impact categories in the concerned country. Additionally, Life Cycle Cost (LCC) is combined with ELP results to give cost estimation of the constructed scenarios. This combination is called Best Available System (BAS) for MSWM. ELP represents the social considerations while LCC represents the economy factor of sustainability.

The empirical results of the multi-dimensional comparison of the production chains including economic, environmental, social and cultural sustainability as well as the overall performance when all dimensions are taken into account simultaneously are presented. In addition, the methodological gaps and future development needed are discussed.

LC02B-1
Evaluating multiple dimensions of sustainability in the case of bioenergy production based on multi-criteria analysis and life cycle assessment
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Environmental impacts of products and services have been traditionally the main focus in life cycle assessment (LCA). Recently, also other dimensions of sustainability including economic, social and even cultural aspects have been received more and more attention in addition to ecological impacts. Multi-criteria analysis (MCA) is a toolbox of methods that evaluate the properties of decision alternatives with respect to multiple, usually conflicting decision criteria.

First, this presentation discusses how MCA can be utilized in LCA when evaluating the sustainability of products and services. The most important benefits of MCA are connected to integration of impact assessments not measured directly in commensurable units and incorporation of subjective preferences into to the assessment framework. Furthermore, the performance of decision alternatives depends on the weights given to various decision elements of the decision hierarchy and the performance of the decision alternatives with respect to the lowest level elements. Different life cycle stages capture the life cycle of the production alternatives from raw material and utilization to end use.

Second, this presentation demonstrate the empirical results of forest biomass based bioenergy production in Finland that was the topic of recent research project funded by the Agency of Technology and Innovation. The project carried out a comparison of the alternative production chains in the methodological framework described above. The analyzed production chains were as follows: (a) Local heat entrepreneurship based on forest chips [Eko energy cooperative]. (b) Wood pellets produced in Finland and distributed to domestic and global markets [Ilomantsi pellet plant]. (c) Direct peat combustion in large combined heat and power (CHP) plants [Fortum CHP in Joensuu]. (d) Biodiesel produced from both forest biomass and peat [Varkaus experimental plant].

The empirical results of the multi-dimensional comparison of the production chains including economic, environmental, social and cultural sustainability as well as the overall performance when all dimensions are taken into account simultaneously are presented. In addition, the methodological gaps and future development needed are discussed.

LC02B-2
Reducing the carbon footprint of the Swiss energy system: which options are most sustainable?
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One of the main objectives of the research project CARMA (Carbon Management in power generation) is the comparative evaluation of potential options for the reduction of Greenhouse Gas (GHG) emissions from the energy system in Switzerland within the framework of future energy scenarios. This assessment is carried out by applying Multi-Criteria Decision Analysis (MCA) methodology. The MCA approach allows considering a comprehensive set of performance indicators addressing environmental, economic and social aspects in decision making and therefore taking into account all three pillars of sustainability; besides these objective criteria, it also allows considering subjective stakeholder preferences, which are used for weighting of the indicators. These preferences are established by a purpose built interactive web interface. The combination and aggregation of performance indicators and preference profiles results in a single performance index for each mitigation option, which can be used for a ranking of these alternatives.

The evaluated GHG reduction options cover the most important economic sectors in terms of GHG emissions: the residential sector, traffic, power generation, and industry. The individual reduction options are compared to a kind of “Business-As-Usual” (BAU) reference scenario until year 2050. This BAU scenario assumes a moderate and steady industrialization and population growth.

Depending on the indicator weighting profile used for the MCA: i.e. on the subjective preferences - the comprehensive evaluation of GHG reduction options can lead to different results compared to the most frequently used purely economic perspective. While specific measures are very attractive from an economic long-term perspective, they can lack individual acceptance, or can be associated with high investment costs as a major obstacle to their implementation. They show the advantages and disadvantages of the evaluated options together with their ranking depending on subjective weighting profiles in a transparent way can facilitate decision processes and help in understanding barriers in the implementation of effective GHG reduction strategies.

LC02B-3
Evaluating of sustainability as Environmental performance of the regional energy systems
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The empirical results of the multi-dimensional comparison of the alternative production chains in the methodological framework described above. The analyzed production chains were as follows: (a) Local heat entrepreneurship based on forest chips [Eko energy cooperative]. (b) Wood pellets produced in Finland and distributed to domestic and global markets [Ilomantsi pellet plant]. (c) Direct peat combustion in large combined heat and power (CHP) plants [Fortum CHP in Joensuu]. (d) Biodiesel produced from both forest biomass and peat [Varkaus experimental plant].

The empirical results of the multi-dimensional comparison of the production chains including economic, environmental, social and cultural sustainability as well as the overall performance when all dimensions are taken into account simultaneously are presented. In addition, the methodological gaps and future development needed are discussed.
LC02B-4
Integrating life cycle analysis, human health and financial risk assessment for the evaluation of contaminated site remediation
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When evaluating remediation technologies for contaminated soil and groundwater, the beneficial effect of the remediation, namely cleaner soil and groundwater, are mostly emphasized without consideration of the environmental and social impact of the remediation activities themselves. Nevertheless, practitioners and decision makers can rely on a broad range of decision tools that can help them to achieve a better balance between economic, social and environmental health aspects of contaminated land remediation. A holistic approach for the management of contaminated land should ideally include an assessment of the environmental risk of the contamination, an assessment of the environmental, social and health impact of the remediation process and a cost-benefit analysis of the remediation project.
A life cycle framework, including a life cycle management (LCM) approach structuring environmental activities and life cycle analysis (LCA) for a quantitative examination, can be helpful for the selection of site remediation options with minimum impact on the ecosystem and human health. Besides addressing the environmental impact of the remediation activities for, attention should also be paid to the consequence of reintroducing a remediate site into the economy.
Moreover, certain soil remediation technologies, especially the more 'gentle' remediation technologies are characterized with a lot of uncertainty with regard to the time frame in which the final remediation goals will be achieved. Unexpected situations can result in an increase of the costs of the remediation project. Therefore, there is a need for practical tools that help practitioners in choosing the correct technology that will not only be effective but also will minimize the financial risk associated with the cleanup.
In the present study, several methods that can be used to estimate the environmental, financial and health impact of a soil remediation process were compared. The case studies worked out in this paper want to provide a basis for a more sounded selection of soil remediation technologies based on human health, environmental impact and financial risk criteria.

LC02B-5
Developments in Social Life Cycle Assessment (S-LCA) for Life Cycle Sustainability Assessment (LCSA) - application to the construction and demolition sector in France
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The third case study focuses on energy saving on the farm, revenues, Trade-offs between yield and environmental impacts are also discussed.

LC02B-6
Life cycle costing of farm milk production - cost assessment of environmental impact mitigation strategies
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Agriculture is a significant contributor to environmental impacts. A study by the University of Arkansas [1] showed that 70% of the carbon footprint of US milk occurs at or before the farm gate. Agriculture is also one of the main contributors to water use and land use, as shown by an ongoing comprehensive milk life cycle study (University of Michigan, 2011). The goal of this presentation is to add costs to the LCA study to determine the impact of the farm milk production system on both the environment and production costs using the same boundaries.

Results and discussion
Three case studies were carried out to compare scenarios from a cost and environmental viewpoint. The first case study describes a change of manure management system to a digester. For a pilot farm located in upstate New York, the global breakdown of the different processes was assessed both in terms of carbon footprint and production costs. Change in GHG impacts and costs are assessed in parallel. Discussion on sources of revenues depending on farm state's location are also discussed.

The second case study compares the cost and environmental impacts of the production of corn, which is one of the major dairy feeds. The costs and impacts (water and land use) are quantified in 3 cases in a sample US state: rainfed, gravity irrigation, and pivot irrigation. This case study provides perspective on how best to represent the costs and environmental impacts, specific impacts categories, indicators and data were searched for and an impacts screening was conducted. Based on sectorial data, it focuses on two stakeholders' categories: workers and local communities. Final results highlight the fact that S-LCA needs strong methodological developments to address intra Europe comparative case studies and to produce methodologies for system definition, data inventory, impacts characterization, reference databases and specific indicators.

LC03 - Increasing scientific and policy understanding through meta-analysis of life cycle assessments

LC03-1
Clarifying estimates of life cycle greenhouse gas emissions from electricity generation technologies: the LCA harmonization project
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Thousands of LCAs of electricity generation technologies have been published. Quality of these studies has varied considerably, as have reported estimates of life cycle GHG emissions. We have completed a systematic review of this literature that included: exhaustive identification and collection of English language LCAs of electricity generation technologies within a well-codified publication type (journal article, report, conference paper, thesis), and multiple, independent reviews of each candidate reference by an interdisciplinary review team that met regularly to ensure consistent screening based on quality of LCA methods, completeness of reporting and modern relevance of evaluated technology.

Estimates varied mainly owing to differences in assumptions of key parameters. It is possible to adjust previously published LCA results to use more commensurate input assumptions and system boundaries. Harmonized estimates of life cycle GHG emissions for six categories of electricity generation technologies: coal, natural gas, nuclear, wind, photovoltaics (crystalline silicon and thin film), and concentrating solar power. While this process is not a formal sensitivity analysis, it does suggest which are the key parameters influencing life cycle GHG emissions for a given technology, such as thermal efficiency for combustion systems, solar resource for solar technologies, and capacity factor for wind turbines, amongst others. Harmonized estimates of life cycle GHG emissions compared to published estimates generally reveal a significant reduction in range and interquartile range from harmonization and often, but not always, an unchanged estimate of central tendency (median).

In this way, harmonization is shown to be a key method that can reduce variability and clarify central tendency of estimates of life cycle GHG emissions of electricity generation technologies in ways that are useful for policymakers and analysts. Given tight harmonized distributions of a large number of estimates, it is concluded that new LCAs of many technologies (e.g., onshore wind, pulverized coal) are not necessary for the purposes of broad policymaking and first-order analyses, while knowledge gaps or uncertainty remain issues for several other technologies (e.g., natural gas, deep offshore wind).

LC03-2
Meta-analysis of life cycle analysis studies on electricity generation with carbon capture and storage
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Currently, electricity generation contributes to 40% of global carbon dioxide (CO2) emissions. Carbon capture and storage (CCS) technology is widely recognized as an
appropriate option to achieve ambitious CO2 reduction targets. In the last decade, numerous life cycle assessments (LCA) on environmental impacts of electricity generation with CCS have been conducted. This meta-analysis comprises fifteen LCAs of the three CCS technology routes (post-combustion, oxyfuel, pre-combustion) for greenhouse gas reduction for different regions (Europe, United States, Japan, global), different fuels (hard coal, lignite, natural gas), and different time horizons (between present and 2050). The purpose of this study is to provide a scientific review of the impacts, their uncertainties and variability. The study answers three key questions: 1) Is LCA an appropriate method to evaluate the environmental effectiveness of CCS, and with what validity or limitations?; 2) Is it possible to draw general conclusions regarding the environmental performance of CCS power plants compared to power plants without CCS from the existing LCAs?; 3) Do certain trends arise across the different capture routes or fuels used? All LCAs suffer from uncertainties and variability in the global warming potential (GWP) of CCS power plants, which may result in an increase in other impact categories, regardless of capture technology, time horizon, or fuel considered. Three parameter sets have a significant impact on the results: 1) power plant efficiency and energy penalty of the capture process, 2) CO2 capture efficiency and purity, and 3) fuel origin and composition. However, the normalization indicates only a small impact from CCS power plants on total global environmental impacts.

The meta-analysis proves that LCA is a helpful tool to investigate the environmental consequences associated with CCS. Differences in the underlying assumptions of the LCAs, and the potential shortcomings and discorindigencies of results. A sophisticated and common understanding of the most important technological parameters is necessary to draw a clearer picture of both single CCS techniques and comparisons across technologies. Therefore, it is essential that LCAs include well documented parameters and describe uncertainties and assumptions precisely. There also remains a wide field of subjects and CO2 capture technologies of 2nd generation (like membranes) that have not been covered yet.

LC03-3
Simplified life cycle approach: GHG variability assessment for onshore wind electricity based on Monte-Carlo simulations
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The environmental impacts of electricity production systems have been widely assessed over the past years with many published LCAs in the literature. In the special case of greenhouses gases (GHG) from wind power electricity, the LCA results variability observed is very high, for example ranging from 2 to 81 g CO2eq/kWh in a literature review performed by the IPCC in 2011. Such result might lead policy makers to consider LCA as an inconclusive method. For environmental impacts from electricity generation this is a sensitive issue.

There is a need for a more comprehensive approach to assess the GHG variability so as to define generic results which meet a general consensus. Different attempts have been initiated in order to address this problematic, the use of meta-analyses in LCA being one of them.

The main objective of this paper is to build a representational model of onshore wind turbines (WT) to assess environmental performances with a simplified life cycle approach.

A first methodology to generate simplified models for WT environmental performances has been designed (reported in the special issue of the Journal of Industrial Ecology) and is now improved with a better identification of the GHG variability assessment. Variability of GHG performances of onshore wind turbines, generated for a representative sample, is assessed through the running of Monte-Carlo simulations to identify the key parameters having the biggest influence on the results. Based on these Monte-Carlo simulations, we plotted GHG performances distributions for two key identified parameters: the WT life time and the annual average wind speed. A set of generic GHG performances curves has been defined as a function of these key parameters. Results are ranging from 2.7 to 119.7 g CO2eq/kWh, a range which is comparable to the mentioned IPCC literature review. These results can be adjusted as a function of either one or both key parameters. This methodology will be applied later for simplified life cycle approaches.

LC03-4
What can meta-regression tell us about variations in life cycle assessment (LCA) results for greenhouse gas (GHG) emissions estimated for advanced biofuels? L. Patouillard, B. Chize, M. Menten, F. Bovart, L. Patouillard
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This paper presents a systematic literature review of LCA studies of advanced biofuels (i.e. biomass-based fuels produced from lignocellulosic materials and microalgae).
The results, notably concerning LCA study robustness, are characterized and estimated using a meta-regression analysis in order to clarify conclusions about their environmental performances. LCA has been widely applied to calculate the Global Warming Potential (GWP is an impact indicator for GHG emissions) associated with biofuel life cycle. Those LCA results can vary significantly depending on various factors: assumptions at biomass production step (N2O emissions estimation, inclusion of direct and indirect land use changes), data for biomass feedstocks, outcomes and choices (system boundaries, impacts categories). This analysis allows to make a first approach of the robustness of LCA on biofuels. Here, we propose an alternative approach to previous narrative surveys of biofuel LCA studies using the meta-regression analysis methodology to describe and synthesizes existing estimates of the GWP of advanced biofuels. It allows to compare and/or combine outcomes of different individual empirical studies with and without similar characteristics that can be controlled for. A database has been built containing a vector of previous studies estimates of GWP (GHG emission indicator expressed in mass of CO2 equivalent per MJ of biofuel) that has been chosen as the dependent variable of the model since GHG emissions reduction is the most important environmental criteria in regulatory texts, and a vector of explanatory variables. These are factors that can influence LCA results and some study characteristics. Advanced biofuel LCA results from peer reviewed articles, research reports (grey literature) and regulatory texts (RFS and RED) are included in the database (41 LCA studies, 85 estimates).

Preliminary results show, ceters parsibus, that the mean life cycle GWP associated with 1 MJ of ethanol, BTL and algae fuel are 27, 21 and 83 g CO2eq respectively (RED fossil fuel reference value is 83.8 gCO2eq/MJ). The analysis shows that GWP estimates are higher in studies that take into account estimate uncertainties, LUC, infrastructures in system boundaries and lower in studies accounting for other impact indicators than only GHG emissions. Moreover, our results indicate that regulatory texts provide lower GHG emissions estimates than peer reviewed studies and that estimates from European studies are statistically higher than American ones.

LC04-5
Sources of variation in life cycle assessments of desktop computers P.L. Teahan, M. Kandlikar
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Life cycle assessment (LCA) studies of desktop Personal Computers (PCs) monitored with the 2006 IPCC methodology were analyzed to assess environmental variability and spatial variability. Assigning a GSD2 to each of the five uncertainty/variability classes ranging from “no” to “very high” uncertainty/variability allows translating the qualitative scales (e.g. watersheds for water use impacts, biomes for land use impacts, and urban/rural archetypes for respiratory impacts, or soil and water archetypes for metal toxic impacts) into the LCA results variability. The uncertainties and spatial variability are quantified and kept separately, allowing for differences in spatial variability of CFs in different spatial scales (e.g. watersheds for water use impacts, biomes for land use impacts, and urban/rural archetypes for respiratory impacts, or soil and water archetypes for metal toxic impacts). The uncertainty and spatial variability are quantified and kept separately, allowing for differences in spatial variability of CFs in different spatial scales.
quantitative uncertainty for each CF using an estimation framework that is consistent with life cycle inventory uncertainty estimates from e.g. ecoinvent. Given the various types and sources of uncertainty contributing to the overall uncertainty of a CF, the estimates provided are not covering all uncertainty sources and types, and might have a somewhat limited accuracy. They are however an important operational starting point to consistently apply uncertainty analysis in LCA, ultimately allowing to quantify the confidence in LCA results and hence the related conclusions and decisions.

**LC04A-2**

**Quantification of uncertainty of characterisation factors due to spatial variability**

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Except for a minority of foreground production sites, elementary flow geographic location is only known to the country resolution. More precision is needed to pick the right spatialized characterisation factors (s-CF) from a set. Countries are usually covered by several s-CF, and their values might span several orders of magnitude. So far, this source of variability has not been quantified. This paper presents a general method quantifying the uncertainty created by the dichotomy in spatial resolution between inventory and impact assessment.

For each country, a generic CF (g-CF) has to be calculated with the s-CF covering it. Two elements are necessary to build the g-CF: the probability density function (PDF) of each s-CF, and a set of weighting factors. The weighting set provides a likelihood of emission on the zone covered by each s-CF, an information lacking in the generic unit processes.

The average and standard deviation of the g-CF is not calculated directly from the weighted s-CF. Instead, the PDF of the g-CF is built by adding the weighted PDF of each s-CF. The average value of the g-CF can be calculated from the PDF and used in deterministic calculations. The standard deviation could be useful if the resulting PDF can be approximated by a normal or lognormal. Otherwise, the full PDF should be reported and used directly in a Monte Carlo simulation.

Finally, the CV (coefficient of variation) of the g-CF can be compared to the CV of the s-CF. The CV of the g-CF will be higher, and this augmentation of uncertainty reflects the spatial variability that cannot be taken into account, due to the lack of spatial information in the generic unit processes.

The impact category chosen to demonstrate this methodology is water use on human health. The spatial delineation of this method is the intersection of countries and water basins, resulting in 808 cells. The likelihood of water use in each cell is based on data from the Watergap model. China is covered by 20 watersheds. The corresponding 20 s-CF are lognormally distributed with CV between 0.6071 and 0.6218. After adding their weighted PDFs, the resulting g-CF is bimodal, with a CV of 7.63. This means that if a good quality water source occurs somewhere in China, without further information on the watershed of consumption, it will be characterized with a g-CF of a value potentially much more or less than with the right s-CF, and roughly 10 times more uncertain.

**LC04A-3**

**Matrix-based sensitivity and uncertainty assessment for evaluating human intake of pesticide residues in food**

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Multiple pathways contribute to human exposure towards pesticides: inhalation after air emissions, ingestion after soil and water emissions and, most importantly, ingestion of directly sprayed food crops. For the latter, we developed a dynamic multicrop model for assessing pesticide residues in food crops and subsequent human intake. However, full uncertainty analysis is still lacking. We already identified half-lives in plants and time between substance application and crop harvest playing a crucial role, but other aspects may also be important.

We designed a simple but accurate regression model with time from application to harvest, half-life in plants, residence times in the environment and key substance properties as input variables. Additional influence can be identified by exploring the wide range of input variables, thereby addressing parameter correlations and defining geometric standard deviations for all relevant input variables as a function of their base uncertainty and spatial/temporal variability.

We studied the uncertainty propagation of the model output by applying a new approach of calculating the overall output uncertainty as a function of the matrix of relative sensitivities of input variables and the covariance matrix expressing their correlations. Model output, i.e. human intake fractions from consumption of food crops treated with pesticides, showed highest relative sensitivities across substances to half-lives in plants and on plant surfaces as well as to the time between substance application and crop harvest. Total output sensitivity is a function of the crop species and is highest for leafy vegetables (lettuce) and fruit trees (apple). We also studied the variability across pesticides, from which we derive crop-specific regression models predicting residues in food crops across pesticides within a factor of 10 of those calculated with the complex model and a function of only a handful of input variables. These simplified models are adequate to assess direct residues for multimedia models used for risk and impact assessment and, hence, enable the user to calculate direct pesticide residues only by providing a very limited set of input information.

**LC04A-4**

**Uncertainty classification and implementation in life cycle impact assessment: application to freshwater ecotoxicity of pesticide application to Maize in The Netherlands**

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How to deal with uncertainty has become a key challenge for integrated assessments. As yet, the application of an uncertainty analysis is not common practice in life cycle assessments. A proper analysis will be facilitated when it is clear which types of uncertainties exist. The aim of this research is to set up a framework to classify levels of uncertainty in life cycle impact assessment (LCA) and demonstrate the practicability of the framework with a case study example on freshwater ecotoxicity caused by pesticide application in The Netherlands.

Three levels of uncertainty were distinguished: (i) Statistical uncertainty, arising from measurement errors, analytical imprecision, and limited sample size; (ii) Decision rule uncertainty, i.e. ambiguity or controversy about how to quantify or compare social objectives; (iii) Model uncertainty, defined as uncertainty about the relations and mechanisms being studied. To quantify statistical uncertainty, uncertainty distributions need to be derived for input parameters and propagated to output uncertainties, e.g. via Monte Carlo simulation. Decision rule uncertainty and model uncertainty can both be made operational with the help of a choice analysis.

A case study that addresses the application of pesticides to maize in the Netherlands was performed to show the application of the various uncertainties for freshwater ecotoxicity assessment. The uncertainty in freshwater ecotoxicity damage scores was quantified by performing a Monte Carlo analysis with chemical-specific input parameters set as uncertain. Decision rule uncertainty was identified by choosing choices in the damage quantification, i.e. availability of toxicity data, choice of effect factor model (linear or non-linear), and the damage factor (modeling up to midpoint or to endpoint). Model uncertainty was quantified by including and excluding pesticide transformation products.

Results showed that damage can vary substantially depending on the value choices made. Parameter uncertainty can increase to a large extent when a more accurate model is applied. Going from midpoint to endpoint applying an uncertain damage factor decreases the damage slightly, but hardly changes uncertainty. Only when transformation products are included, going from midpoint to endpoint increases median damage clearly.

**LC04A-5**

**Data quality metrics in Life Cycle Impact Assessments: application to a case study of cellululosic biofuel**

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Although guidelines exist for assessing data quality in life cycle impact assessment (e.g., ISO-1404), they are seldom implemented satisfactorily in practice. A need therefore exists for transparent, consistent data quality metrics that facilitate the prioritization of data needs and enhance the reliability of LCAs. Monte Carlo (MC) methods, such as sensitivity and uncertainty analysis are used to identify the most influential inputs and to understand how input uncertainty (variability and lack of knowledge) is propagated through to the calculation of output metrics. However, MC methods are rarely applied in LCAs. Here we demonstrate the application of sensitivity analyses coupled with a data quality evaluation scheme, to assess uncertainty in output metrics in our LCA "Carbon Tool", which quantifies the environmental and human health impacts of 150 bioenergy pathways from national-scale cellululosic biofuel production. Program output, i.e., the Carbon Tool estimates environmental and health impacts associated with biomass, biorefining, and transportation stages of biofuel production. Specific output metrics include: greenhouse gas emissions (fossil CO2, and GW100), total energy, and human health impacts associated with criteria air pollutant emissions. We use the Spearman rank correlation coefficient to identify influential variables for each output metric, with specific application to Mosanath ethanol production. We then evaluate the uncertainty in influential system variables, using a qualitative data quality evaluation scheme adapted from Junnila and Horvath (2003). Of the eight variables evaluated, Combined Heat and Power (CHP) penetration was the most influential system variable set as uncertain. Decision rule uncertainty was determined by identifying choices in the damage quantification, i.e. availability of toxicity data, choice of effect factor model (linear or non-linear), and the damage factor (modeling up to midpoint or to endpoint). Model uncertainty was quantified by including and excluding pesticide transformation products.

Results showed that damage can vary substantially depending on the value choices made. Parameter uncertainty can increase to a large extent when a more accurate model is applied. Going from midpoint to endpoint applying an uncertain damage factor decreases the damage slightly, but hardly changes uncertainty. Only when transformation products are included, going from midpoint to endpoint increases median damage clearly.

**LC04A-6**

**Uncertainty analysis in macro-level life cycle assessment**

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The Netherlands.

How to deal with uncertainty has become a key challenge for integrated assessments. As yet, the application of an uncertainty analysis is not common practice in life cycle assessments. A proper analysis will be facilitated when it is clear which types of uncertainties exist. The aim of this research is to set up a framework to classify levels of uncertainty in life cycle impact assessment (LCA) and demonstrate the practicability of the framework with a case study example on freshwater ecotoxicity caused by pesticide application in The Netherlands.

Three levels of uncertainty were distinguished: (i) Statistical uncertainty, arising from measurement errors, analytical imprecision, and limited sample size; (ii) Decision rule uncertainty, i.e. ambiguity or controversy about how to quantify or compare social objectives; (iii) Model uncertainty, defined as uncertainty about the relations and mechanisms being studied. To quantify statistical uncertainty, uncertainty distributions need to be derived for input parameters and propagated to output uncertainties, e.g. via Monte Carlo simulation. Decision rule uncertainty and model uncertainty can both be made operational with the help of a choice analysis.

A case study that addresses the application of pesticides to maize in the Netherlands was performed to show the application of the various uncertainties for freshwater ecotoxicity assessment. The uncertainty in freshwater ecotoxicity damage scores was quantified by performing a Monte Carlo analysis with chemical-specific input parameters set as uncertain. Decision rule uncertainty was identified by choosing choices in the damage quantification, i.e. availability of toxicity data, choice of effect factor model (linear or non-linear), and the damage factor (modeling up to midpoint or to endpoint). Model uncertainty was quantified by including and excluding pesticide transformation products.

Results showed that damage can vary substantially depending on the value choices made. Parameter uncertainty can increase to a large extent when a more accurate model is applied. Going from midpoint to endpoint applying an uncertain damage factor decreases the damage slightly, but hardly changes uncertainty. Only when transformation products are included, going from midpoint to endpoint increases median damage clearly.
Introducing an uncertainty analysis methodology, in an international carbon footprint accounting for decision making, Renault Group case study

The development of life cycle assessment (LCA) has led to new life cycle thinking applications such as the macro LCA approach (M-LCA), an extension of consequential LCA (C-LCA) that implements elements of prospective LCA in order to model medium- and long-term environmental impacts related to global economic perturbations caused by major changes occurring in multiple life cycles. In M-LCA, economic impacts are based on the GTAP model, an economic general equilibrium model (GEM), which provides for various economic conditions for each sector in every region of the world in response to a given economic change. The environmental impacts of each regional economic sector are then computed according to the LCA methodology. As uncertainty related to GEMs and LCAs is recognized as significant, the uncertainty associated with M-LCA is explored in this paper. In order to address this issue, an uncertainty analysis was conducted on an M-LCA comparison of two European Union (EU) energy policies (business as usual vs. bioenergy). Uncertain exogenous variables and internal parameters used by GTAP were combined in order to develop 27 scenarios that were run in GTAP to define a tree of potential economic consequences caused by each EU policy under the assumption of each scenario. Environmental impact uncertainty for the 27 scenarios was then assessed using a Monte-Carlo (MC) analysis. Results of the uncertainty analysis show how the M-LCA comparison of EU policies is not sensibly affected by uncertainty for climate change and paper manufacturing resources while the comparison is more uncertain for human health and ecosystems. The uncertainty analysis also highlights that several sources of uncertainty in M-LCA cannot be assessed due to lack of information on data uncertainty (external data used for the linkage of GTAP and LCA databases and forecasts used to model the evolution of temporal variables) and the difficulty in comparing GTAP results with historic data. Additionally, it appears MC analysis is not adequate to study uncertainty propagation in complex models such as M-LCA. Indeed, due to the significant uncertainty of uncertain parameters, the time required to conduct the MC simulations makes this approach too computationally expensive for extensive computational resources. Therefore, the development of another approach based on an uncertainty management method more suitable for large models, like Gaussian quadrature or Fourier transformations, would improve the management of uncertainty in M-LCA.

LCOB-1

Technological, geographical, time-related uncertainty measurements for textile spinning and weaving processes

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Life Cycle Assessment (LCA) is a very relevant tool on the quality, relevance and reliability of the Life Cycle Inventory (LCI) data sets selected by the LCA-practitioner. When modeling a system, the representativeness of the LCI data set is complemented by the representativeness of the data set in the context of the specific system. The representativeness characterizes, in how far a data set in a system model represents the truly required process of production. According to the ICLC handbook [1], the use of not fully appropriate data is justifiable only if this is not relevantly changing the overall LCA results compared to using fully representative data; otherwise the lower achieved representativeness shall be documented in the report. But when the required data is missing the practitioner has limited possibilities to evaluate any differences between the required data and the available data.

This paper aims at evaluating the representativeness of different LCA-practitioner choices regarding the geographical, technological and time related representativeness in the modeling of a product life cycle. The examples are taken from two technologies from the same industry domain that behave differently and require different LCA methodologies.

First, different LCI data sets for spinning and weaving processes in the textile industry are inventoried. The precision, completeness, representativeness and methodological consistency of these LCIs are briefly described. Then, the following data quality tools: gravity analysis and sensitivity analysis [2] are applied at the assigned LCI results. The gravity analysis reveals the main contributors to indicator scores. The sensitivity analysis measures the change in the indicator results for induced changes in LCI results. Practically, the LCI results for different geographical situations, for different technologies (time related or not) and for different scenarios (average world scenario and worst case scenario) are compared.

LCOB-2

A comparison of uncertainty propagation methods in an LCA study

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Several methodologies can be used for defining and propagating uncertainties affecting model input data in LCA studies, according to the nature of available information. Probabilistic methods are the most commonly used and consist in defining uncertainties as single probability distributions, and then propagating them through stochastic modelling or analytically. For cases where available information does not justify the use of single probability distributions for representing uncertainty, possibility theory provides an alternative method which consist in defining uncertainties as fuzzy sets.

The objective of this paper is to illustrate the fundamental differences between these approaches using on the one hand classical stochastic modelling, fuzzy calculus, and finally a hybrid method which combines both approaches. In this last method some parameters are represented by single probability distributions (because they are justified by available data) while others are represented by fuzzy sets (because available data is incomplete and or imprecise). The joint propagation of these different modes of uncertainty representation was performed using the independent random set method proposed by [1].

The case study investigates the benefits of sending the organic fraction of household waste to anaerobic digestion instead of incinerating it, in Danish conditions. For each chosen parameters, data was gathered through a literature review to define the uncertainties underlying each parameter, using either a single probability distribution representation, or else a fuzzy-set representation. Comparison between the three propagation methods illustrates the very conservative nature of the purely fuzzy calculation, of which the result of the stochastic calculation is but one representative among others. Results of the hybrid calculation on the other hand are more precise than the fuzzy calculation but of course less precise than the purely stochastic result which assumes that single probability distributions are perfectly known for all input parameters.

In real-world situations of LCAs, available data are typically of different natures. Therefore the proposed method can serve to jointly propagate the different types of uncertainties in the LCA.


LCOB-3

The impact of processing natural resources on uncertainties in life cycle assessment

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Natural resources are often connected to variable compositions. Ore from mining operations seldom provide the same ore concentration of their production everyday and the further processing has to deal with it. The same model is visible in renewable resources like wood, grass, and crops for example. By processing these materials the processes has to be either adapted to the changing quality of input material or it is run on average level. An average process control level may have an inefficient effect on the process quality.

This presentation will concentrate on the feature: How to run a material and energy efficient production process with less environmental impact? Our study case is the production of pig feed in a commercial feed processing plant in Northern Germany. Our aim is to relate the energy consumption not only to the process steps but to the various recipes. By identifying the carbon footprint for each recipe we are going to optimize the energy input into the process without influencing the product quality. This is our highest challenge, since the quality of the end product cannot be changed due to uncertainties that can not be influenced by the process control (chemical composition)

The development of the expert system of the production process (case study pig feet) is performed according to uncertainty management. Uncertainties are present at any time in the process and has therefore taken into account during the decision making process. In the end it will be possible to simulate the production process with varying parameters to evaluate the result of the simulation. This helps in assessing the process set up and in logistics question (what is the best cyclic order of the recipes to consume the least energy?).

LCOB-4

Introducing an uncertainty analysis methodology, in an international carbon footprint accounting for decision making, Renault Group case study

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Climate change and energy dependence are major challenges for the transport industry. Tackling these requires major decisions and therefore to integrate Greenhouse Gases
The majority of the carbon footprint (85%) calculations are in the “good”level, while 10% are of “middle”uncertainty and finally 5% are evaluated as “very good”. When making a decision, it is important to allocate budget toward the most important topics. This uncertainty approach enables decisions to choose under the best conditions. The risk factor can be evaluated and improve the inventories each year. All uncertainty factors are based on Dronton expert judgment and should be challenged by external experts (eg. Quality of background LCI database).

Implementing Carbon Footprinting is a major step towards an Environmental Footprint management by introducing the life cycle perspective and involving the whole company. Nevertheless such a wide perimeter implies a complex calculation protocol and the uncertainty calculation will have a huge importance to tackle the complexity of this management and implement the best decisions.

Finally, the authors stress that even if uncertainties exit, these shall not be a barrier for taking action.

**LCOM-5**

Uncertainty and variability in the carbon footprint of U.S. coal-fired power production

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The overall carbon footprint of power generation can be calculated using life cycle assessment (LCA). For most LCAs, average conditions are used to estimate the environmental impacts of a process or product. However, regional and technological differences in product manufacture, use, and disposal may cause specific life cycle impact (e.g. GHG emissions) to be lower or higher than that of an “average”life cycle. Moreover, uncertainty in process data used to model life cycle stages may affect the accuracy and precision of life cycle impact assessments. Whereas uncertainty is defined as lack of knowledge and can potentially be reduced by additional research, variability is an inherent characteristic of supply chains and the environment and cannot be reduced.

The aim of this study is to quantify the contributions of uncertainty and variability to the range of the carbon footprint associated with coal-fired electricity production in the U.S. This is achieved by considering the variability of key factors such as distances between coal mines and the power plants that supply them and power plant efficiency. We also characterize parameter uncertainty, e.g. the methane emission associated with a coal basin. The LCA included three stages: mining, transport and power generation, and utilized a functional unit of 1 kWh of electricity generated at the plant. Parameter uncertainty was quantified via Monte Carlo simulation, whereas variability was taken into account via the efficiencies of power plants and coal transportation receipts reported by the U.S. EIA.

We presented independent carbon footprints for U.S. coal power plants with uncertainty ranges. Our results show that the variability in electricity plant efficiencies particularly lead to a large overall variance of the life cycle emissions of coal power. Results indicate that improvements in inventory data and assessment parameterization reduce the variance of the life cycle emissions. The results can be used to assist decision-makers in ranking the carbon footprints of coal plants and facilitate the comparison of coal power emissions with other power generating technologies on a statistical basis.

**LCOM-6**

A protocol for approaching uncertainties in life-cycle inventories Monte Carlo analysis - a practical example using aquaculture feeds

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Although LCI values often are presented in absolute numbers, uncertainty and variability are common traits of both foreground and background data. Uncertainties result from limited temporal, spatial and technological coverage of both economic and environmental flows, with a direct influence on results. In LCIs of food production systems, however, the influence of variability (ontological uncertainty) may be increasingly predominant as production is governed by natural fluctuations (e.g. yields). Outcomes of LCAs results describing the same production system may therefore vary with up to an order of magnitude.

Aquaculture feeds are here used as a practical example to demonstrate the proposed approach to uncertainties in LCIs. The modelling includes foreground data collected from feed mills in Asia and literature sources, while background data derive from the ecoinvent v 2.2 database. Each processes within the system boundary was approached using a standard way to assign mean, standard deviation and distribution to most economic and environmental flows. Each of these variables were based either on primary data, weighted averages derived from a meta-analysis or a numerical unit spread assessment (NUSAP) pedigree. The inventories were later simulated using a Monte Carlo analysis to generate confidence intervals for individual flows.

Using relevant up-to-date inventories is crucial for achieving accurate results and taking uncertainty into account is the only way to justify these results. We, therefore, herein present a practical way on how to consistently source and analyse inventory data using a mix of real values, weighted averages and NUSAP estimations. This allows for a wide range of data sourcing and a constructive strategy for highlighting areas of great uncertainty and identifying data gaps.
How to correct price for monetising non-renewable resource consumption?

In poteable water production, the question of finding a fair trade-off between the water quality increase, from raw water to at least the potable water standard, and the total effort to be spent to reach that objective is very common despite not trivial. Few comparative LCAs of potable water plants have been published, mostly disregarding the water quality increase in the functional unit and therefore obtained erroneous conclusions. Water quality gain (WQ) can be evaluated by using water quality indicators as the operational characteristics for Water (SFOEs) and, through a statistical approach, regarding the total effort spent to reach the gain (TE), monetisation of LCA results (Stepwise2006 and Eco-costs2007) is indeed a very effective and practical approach, as compared to traditional single scores like ReCiPe, to obtain a single numeraire, which can be further combined to the total operational costs of the water treatment plants to be fully understandable by investors and decision makers. In the framework of an international research project, we developed a novel performance index (PI), defined as the ratio WQ/TE, which allows to properly compare alternative potable water production plants. Statistical tests are used to get a confidence interval for the PI of each plant but also for the difference of the PIs between plants. The PI was applied on two existing treatment plants managed by Suez Environment in France. The plant comparison using the PI calculated with ReCiPe is not conclusive, since the confidence interval of the difference of the average PIs includes zero (too high uncertainties). The same evaluation with monetized scores, augmented by operational costs, gives a clear preference for one of the two sites. Further research is ongoing to add infrastructures data in the LCA calculations and to implement LCA uncertainties into the performance index.

LC06-5

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Monetising aims at reflecting how human well-being is affected by an activity. This paper focuses on the impact category “Non-renewable resource consumption” (mineral and fossil). The cost for the society of consuming such resources corresponds to the well-being loss due to the increased resource scarcity in the future as a consequence of its consumption today. It can be assessed through the opportunity cost, defined as the welfare loss (the cost) of being deprived of an extra unit of resource for its most valuable use, i.e. the one providing most utility. In a perfect market, the price is optimal as it allows maximising the total welfare provided by the resource (optimal resource allocation).

In a more realistic market, the question is: “Does the actual market price need to be corrected for deriving the cost for the society of consuming resources?” The methodological approach developed is based on the following analysis:

- The market price is driven away from the optimal price since, as generally admitted, private discounting rate is higher than social discount rate.
- Other main drivers in market pricing have similar mechanisms in actual and perfect markets and, furthermore, these drivers are better assessed by the market than by a modeller.

The developed monetisation factors for non-renewable resource consumption consist hence in using trend market prices multiplied by a factor correcting for the excessive effects of potential future internalisation (i.e. effects of potential future internalisation are not taken into account).

In practice, when assessing for example various end-of-life scenarios for electric and electronic waste, the total monetised impacts obtained for all impact categories are often significantly reduced compared to the private values.

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LC06-4

Development of an integrated indicator for land use based on ecosystem services

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In life cycle impact assessment (LCIA), potential impacts due to land occupation are calculated with the equation: Iocc,i = surface * time * ΔQi. Currently, the only i indicator for the land loss ΔQi is related to terrestrial biodiversity (PDEMD;year) and is not representative of all impacts, which are described by the European Commission (2010).

Recently, an improved land use method has been developed with the UNEP/SETAC Life Cycle Initiative project (LULCIA, 2008-2011). This method relates land use to six new indicators: biotic production (BPP), erosion regulation (ERP), fresh water regulation (FWRP), mechanical and physicochemical water purification (WPP) and carbon sequestration (CS). These indicators represent provision and regulation services, as defined in the Millenium Ecosystem Assessment (2005).

While this development improves LCIA methodology, it also reduces the decision support system capacity of LCA, providing seven midpoints for land use alone. This project aims to develop a new method (factors in $/indicator) uniting economic valuation as a common thread to convert the new indicators to endpoints representing ecosystem services loss (potential damage costs). This conversion also allows aggregating into a single indicator. The values of BPP and CSF are respectively estimated with proprietary software, which is not yet available in open edition. The other regulation services are calculated through current tax rates. The others, as they are considered essential (conservation approach).

As a local impact category by nature, spatial variability and the availability of the compensation systems are taken into account. Economic valuation assumptions will be discussed. Finally, this method brings a new level of interpretation and potentially allows LCA to assess other impacts related to land use, such as aesthetics and recreational aspects.

This method will be applied on a case study involving the comparison of production locations for bio-based polymers.


LC06-3

How to correct price for monetising non-renewable resource consumption?

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Monetising aims at reflecting how human well-being is affected by an activity. This paper focuses on the impact category “Non-renewable resource consumption” (mineral and fossil). The cost for the society of consuming such resources corresponds to the well-being loss due to the increased resource scarcity in the future as a consequence of its consumption today. It can be assessed through the opportunity cost, defined as the welfare loss (the cost) of being deprived of an extra unit of resource for its most valuable use, i.e. the one providing most utility. In a perfect market, the price is optimal as it allows maximising the total welfare provided by the resource (optimal resource allocation).

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LC06-2

Mainstreaming life cycle management: using a sector based and regional approach in Northern France in the textile, seafood, packaging and mechanical sectors

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Nestlé, the World's leading Nutrition, Health and Wellness Company, is committed to environmentally sustainable business practices. We assess the environmental impacts of our products using LCA and ecodesign tools. While LCA has been shown to be useful for claims and communication of the performance of our products to third parties and the public, the timeline and cost of conventional LCA is not practical for a widespread application throughout the company.

PQET, a packaging ecodesign tool, is being used to assess the environmental performance of packaging systems. Its low cost and rapid results generation allow for it to be systematically applied throughout all packaging innovation & renovation projects at Nestlé, creating knowledge on environmental impacts of our packaging and promoting innovation to develop new packaging systems with reduced environmental impacts.

Building on the success factors of PIQET (pre-defined goal & scope and inventory database, simple to use, fast result generation), we have developed a new ecodesign tool that will take into account the entire life cycle of a food product, including the production of ingredients, processing, packaging, distribution, consumer use, and end of life. To further accelerate the use of such a tool, a link with the company recipe and trial management system has been established. By selecting an existing recipe or trial of a new product from the management system, the ingredients specifications, quantities, and the corresponding processing steps can automatically be uploaded to the ecodesign tool, significantly reducing the manual work required to complete the assessment. By integrating the outcomes of the ecodesign assessment into the stage-gate process for product innovation, a systematic evaluation and optimization of the environmental impacts of all products can be achieved.

For claims and communication of tool results to the public, however, an independent peer-review process according to ISO 14040 & 14044 will still be required. To simplify this process, independently established LCA and ecodesign methodologies (e.g. the ecoinvent database) are used. Furthermore, transparently, independently generated LCI data is incorporated into the ecodesign tool. This reduces the possibility for tool users to introduce bias into their studies and increases the reliability and credibility of the ecodesign tool.
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Whilst Life Cycle Management is becoming commonplace in larger corporations, or forward thinking governments, it is far from mainstream. To achieve sustainable production and consumption patterns, LCM needs to be taken up by whole supply chains that include many small and medium enterprises. These businesses typically lack the financial capacity or human resources to implement LC tools on their own, and are wary of working with support organisations outside of their sector or local area.

In Northern France, the textile, seafood, packaging and mechanical sectors are developing strategic action plans to integrate Life Cycle Approaches (including ecodesign and product environmental labelling) into businesses, education and research organisations. Each study is led by a competitiveness clusters or technical centre working within the sector.

The paper will present results of the benchmark of life cycle initiatives and tools relevant to each sector, and assessment of the existing capacity of businesses, education bodies and research centres in the region. Focus will be given to how potential actions are prioritised through engagement with key stakeholders (such as businesses, universities and professional federations) and how this inclusive process maximises with success factors and helps overcome barriers to implementation.

The four action plans are developed in parallel, enabling the sectors to identify cross cutting actions. The paper will explain how this process is managed through a network of “Life Cycle Champions”. Champions have been trained in the eight sectors; and meet regularly to exchange experiences in implementing life cycle approaches and identify cross cutting projects. The 4 other sectors will undertake the strategic action planning process in a second “wave”, to incorporate learnings from the first process. This innovative approach to mainstreaming LCM leverages sectoral and regional networks to help overcome barriers to implementation. From a business perspective, integration with existing professional organisations means that SMEs access advice and tools through organisations that they already know and trust. Working with several sectors in parallel through the Life Cycle Champion network encourages a multidisciplinary approach, essential to improving decision making across entire supply chains.

The automotive industry is facing major challenges and the product design is strong in order to reduce production cost and usage value within the capacity of the nature to support the mobility growth. This goal will be achievable only when a co-innovative product design will be set up, involving the whole value chain [1]. This is an ambitious target and this paper will show how life cycle assessment evaluation tool could be a major contributor to bring the various parties altogether.

Several example are identified, from the more obvious which is the critical review during which the practitioner will open his work to Research Institutes / Universities or Environmental NGOs. No盖论性Organisation. Regarding the impact assessment, reliability can be worked with Research Institutes when comprehension can be studied with Customers. Concerning Inventories employees and suppliers will work hand in hand and finally for the scope definition, this will be a good opportunity to involve the customers in order to define the relevant functional unit. This last point is particularity true when speaking of the electric vehicle, a new technology and potentially a major change in people habits of buying and consuming their mobility.

One of the next steps is to refine the definition for electric vehicles LCA [4]. The main difficulty [5] is to define a function equivalent between thermal and electric vehicles. The ILCD handbook [2] recommends a functional unit define by four items: What, How much, How long and in What way. The reason for any doubt take place in the last item, in what way. This question was studied under two approach, one “product centric” based on functional analysis and one “people centric” based on customer (emotional) needs. Then we can enlarge the main differences between the thermal vehicle strengths - reassurance and aesthetic - versus electric vehicles ones - environmental friendly and fun. Therefore, equivalent functional units shall add a reassurance dimension to the electric vehicle functional unit such as an easy access to a “long autonomy range” vehicle when needed.

This experience on the electric vehicle shows also that LCA can be a very useful tool to bring the stakeholders (unusual ones as NGOs as well) around the table with a positive and constructive scientific approach.

LCT in the floor-covering industry: the strategy of Tarkett

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Tarkett is world-wide leader in innovative flooring and sports surface products, providing integrated solutions to professionals and end-users. Despite its long time concern about environmental issues, actions remained not harmonized so far. The main tool reflecting environmental performances of Tarkett activities and products was the regular publication of five environmental “Key Performance Indicators” on global production. The need for a comprehensive sustainable development strategy to be integrated in the daily practice has emerged and Tarkett is now engaged with a multifold challenge. To tackle this endeavor, Tarkett has chosen the support of the Public Research Centre Henri Tudor. Life-Cycle Thinking (LCT) has been identified as main approach, since the products are at the core of the environmental strategy system. Tarkett deals with a wide customers base, 30 worldwide production sites, 8 families of products, and therefore a wide range of raw materials. The implementation of LCA as a fact found based practice for process and product environmental improvement has therefore become a priority. Although LCA is used as an eco-design driver and a strategic tool, the company is aware that this tool is not sufficient at its actual level of development to address some specific issues. Therefore Tarkett is engaged in complementary development studies and processes. The adopted approach is a progressive integration of LCA as a decision-making tool in a coherent way for all Tarkett’s branches. First Tarkett acquired the methodologies through training courses in Europe and the dedicated IT software is also being developed to facilitate the access to LCA models and allow Tarkett professionals to run ecodesign simulations. The positive feedback from this test integration phase convinced Tarkett of the ability and reliability of environmental strategy at the decision making level. Further development phase is now launched; the vision is to combine the complementary tools already used by Tarkett in the LCT process, in order to set a robust decision making based on eco-innovation criteria.

LCE-6

Promoting use of life cycle management in Finnish companies - challenges, benefits and suggestions for future value networks

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Life cycle thinking (LCT) and life cycle assessment (LCA) along with other life cycle (LC) methods are important tools in assessing environmental impacts of products and services, and to support environmental decision making in companies. Use of those methods can also help companies to find business opportunities by taking precautionary actions. This study presents the main drivers and barriers for companies to apply LCT, LCA and other LC methods. We also suggest a roadmap for the promotion of these methods in companies and their value networks. We first assessed the methodological challenges of the LCT, LCA and other LC methods from the companies perspective by interviewing experts. An awareness gap between the majority of those tools was assessed in several case studies. The assessments were based on the data from literature and companies. Several stakeholder workshops were organized to get practical views on the use of LC methods. The current and future use of LC methods in companies was also studied using an internet questionnaire and thematic interviews. They revealed that the companies can coarsely be categorised in four main company types regarding their knowledge, resources and potential to utilize life cycle methods in practice. The findings were supported by the case studies. Three company types were named as ‘interested outsiders’, ‘learners’ and ‘forerunners’. Additionally a large group of companies are not aware of the use of life cycle methods and their potential benefits. The main drivers for the environmental management of companies included legislation requirements, improvement of cost-efficiency and customer requirements, among others. A large number of
companies are not familiar with life cycle methods and their potential benefits. Additionally, especially of small and medium-sized enterprises (SMEs), often have no temporal, human and economic resources to learn and educate themselves on the benefits of using LCT and LCA. The main challenge related to wide use of LCT and LC methods in companies is probably related to the communication problem between the LC researchers and companies, and therefore much more practical approach need to be used when spreading LCT in wide scale. The next step in promoting the use of LCT and LC methods in decision making in practice will be a pilot project, in which all the actors within a region, value-chain or sector will be introduced to work with each others in a networking process, including special type of training for SMEs.

LC07 - The UNEP-SETAC Life Cycle Initiative: a decade of supporting the global LCA community

LC07-1
Scientific legacy of the UNEP-SETAC Life Cycle Initiative: contributions and significance
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The UNEP-SETAC Life Cycle Initiative is a joint venture formed in 2002, among several purposes, to foster the development, validation, and dissemination of methods, tools and guidance to support sustainable consumption and production to the world. Over its ten year existence, the Initiative has conducted dozens of projects which have contributed to the efficiency, effectiveness, and rigor of life cycle approaches on a global scale. This presentation will address those contributions from the viewpoint of meeting critical needs for maintaining and enhancing the quality of the science underpinning life cycle approaches. It will also place those developments in the context of emerging research and practice from academic, government and business organizations, providing a perspective on the significance of the Initiative's contributions to the science of LCA.

LC07-2
Top 10 points about life cycle every government decision maker should know
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ECCO GLOBAL S.A., San jose, Costa Rica
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Top 10 bullets that government officials should remember about Life Cycle Approaches when considering sustainability decisions is a contribution of the UNEP/SETAC Life Cycle Initiative to the discussions for the next UN World Summit for Sustainable Development. The Initiative has successfully responded to the call from governments for a life cycle economy as stated in the Malmö Declaration and contributed to the development of a 10-year frame work of programs on Sustainable Consumption and Production (SCP).

"To bring science-based life cycle approaches into practice worldwide" is the Mission of the Initiative and 3 objectives were established to achieve it: enhancing the global scope of life cycle data and methodologies through research and innovation; facilitating the use of life cycle approaches worldwide by business, governments and the general public; and expanding capabilities worldwide to apply and to improve life cycle approaches through education and training. The top 10 bullets were disseminated for the first time amongst government representatives during the High-Level CSD Intercessional Meeting on SCP on 2011 as:
- Every product causes environmental impacts in its supply chain, during its use and in its disposal
- To improve and preserve the world, the different impacts of products need to be identified and quantified
- Life cycle assessment is the only standardised international tool for identifying and quantifying impacts of products
- By identifying, impacts can be understood and managed by companies and governments
- Environmental impacts affect ecosystems in many different ways
- Ultimately, all environmental impacts affect humans
- Companies and the decision of governments must be made based on which impacts are most important and which should be addressed first
- Impact of impacts depends on your perspective
- All life is connected, how those connections are managed is up to you
- When making sustainability decisions, you must consider the entire life cycle including trade-offs

While the first five bullets introduce key concepts the sixth bullet highlights the strong linkage between the environment and human beings. The last four bullets compiled the message of life cycle SECD data and methodologies for decision makers.

LC07-3
Strategizing capacity development for life cycle management - cases from Vietnam and Malaysia
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The promotion of life cycle management in companies in developing countries has to build upon capacity to perform and interpret life cycle assessments. Drawing on two case studies: one on pangasius aquaculture in Vietnam and the second on palm oil production in Malaysia, the paper identities - in these two different national contexts - current capacity constraints and future options for strategizing efforts, which target transition towards sustainable production and consumption. Both cases point to the need for capacity to collect valid and reliable data. New life cycle inventories (LCIs) have to be built to reflect real processes in the main sectors of the national economy. While subsidiaries of foreign owned companies take the lead in adopting a life cycle approach in environmental management as a result of corporate policies, the large sector of medium and small scale companies have few or no incentives in their national context at a time when export markets introduce conditionalities concerning sustainable production practices. The case studies also show that most examples of LCA practiced primarily seem to be motivated by concerns about the public image of a particular product in the market place. The most significant driver currently seems to occur, when a vital product in the export profile of a developing country is contested on the world market for its environmental impacts. In conclusion, to move beyond the awareness of life cycle thinking and the communication of "greener" brands, the linear model of rolling-out and implementing LCA practices in developing countries, which basically is derived from requirements of the LCA methodology itself, the needs revision. Enabling and data resources, facilitating communication and training, providing limited support for application, and creating international networks for research definitely contributes to capacity development. However, a coherent strategy - drawing on the paradigm shift initiated by UNDP in 2002 and subsequent research on the export markets for its environmental impacts - is needed to identify options for international support of stakeholders, for entities in the enabling institutions, for foreign investors, and for regulatory authorities in economic and industrial policies and foreign trade relations.

LC07-4
Water use in life cycle assessment and water footprinting: outputs and prospects of the working group WULCA
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Water use has been largely neglected in LCA of the first decades. While some water use data has been collected in inventories, environmental impacts related to use and consumption of this vital resource have not been addressed until 5 years ago. At this time different research groups have started to develop methods to quantify impacts, and the UNEP-SETAC life cycle initiative has established the working group for water use in life cycle assessment (WULCA).

The group is organized by a core of researchers and practitioners actively contributing to the development of the field, while a larger group is involved in rounds of feedbacks and invited to meetings in order to allow input from various stakeholders in a very vast and dynamic field. The deliverable of the group was to compile a consistent framework for addressing the environmental impacts related to the various types of water use and areas of protection affected. Another challenge was to establish a common terminology. As a result, the core of the group published a scientific article which builds the basis for future research in this area. In a second phase, the group focused on comparing different available methods, comparing their scientific quality, completeness and applicability on order to provide guidance to LCA practitioners and other interested people about the status of inventory data and impact assessment methods. A scientific paper will be submitted in December 2011.

The working group is also involved in the standardization process of water footprinting, cooperating with research partners and practitioners outside the LCA community in order to contribute to a standard that is compliant with the LCA perspective.

Future action points are the harmonization of existing LCA methods and inventories. Building up on the methodological review, quantitative comparison of different approaches targeting similar impacts are performed to derive estimates of robustness, while research of missing impact pathways is fostered. Inventory data collection has
been of lower priorities so far, however, with increasing demand for both robust water use information and related impact assessment, the supply of representative water use data it becomes a relevant issue, especially due to the high spatial variability of water use and related impacts.

LC07-5
Value of a Life Cycle approach in evaluating the environmental impacts of packaging for food and beverage applications
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An articulation of the benefits of the life cycle approach to design, manufacturing, use and end of life management of packaging for food applications is important to further examination of the role of packaging within the nexus between safety and security of packaging in food and beverages and environmental protection. Key items of focus for the research and analysis included, but not limited to:
- What is the value of a life cycle approach for beverage and food products and packaging?
- What is the value of including all life cycle stages in evaluating the packaging/food systems to reduce overall life cycle impacts?
- What is the value of including multiple impacts in evaluating the packaging/food systems to reduce overall life cycle impacts?
- What is the value of understanding the food and/or beverage industry as a whole in an evaluation of the packaging life cycle impacts?
- What characteristics of future LCA studies should be considered when evaluating the food/packaging life cycle?

Examples of how the waste management hierarchy and LCA results interface/connect
This presentation will present the results of study to examine the Value of a Life Cycle Approach in Evaluating the Environmental Impacts of Packaging for Food and Beverage Applications.

RA01-1
Applying models to risk assessments: from the organism to the ecosystem level
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Toxicity of chemicals is measured on many individual endpoints: feeding, growth, reproduction, and survival. The sensitivity of these endpoints of model species to chemicals is used to gauge the negative effect these chemicals will have on higher levels of biological organization such as populations. However, when using different individual endpoints to estimate risk at the population level, it is important to know the relative sensitivity of populations to changes in different endpoints. For example, is a 20% reduction in feeding more harmful to a population than a 20% reduction in reproduction? In part, these questions have been addressed via "elasticity analyses" using mathematical methods. In elasticity analysis, the sensitivity of population growth rate to changes in different generalized modes of action (reduction in survival, reproduction, or growth) is calculated. While these provide a useful estimate of relative sensitivity of one important aspect of populations, intrinsic growth rate alone does not define the dynamics of populations. Most population dynamics are driven in some way via an interaction with their prey species, and a change in the dynamics of individuals may alter this interaction in a way not described by the population growth rate.

To overcome this problem by using a method which considers the interaction of the population and their prey and their dynamics in time by using an individual-based model (IBM). Specifically we use the recently developed framework DEB-IBM (Martin et al. in press). The DEB-IBM framework is an implementation of the Dynamic Energy Budget (DEB) theory in an individual-based modeling context designed to extrapolate from individuals to populations. In this framework DEB provides the rules for how environmental conditions (food, temperature, toxicants) are translated into individual performance (growth, reproduction). DEB theory is generic, as differences between species are described via different parameter values within the same model framework. Using DEB-IBM (http://cream-inn.eu/projects/wp-1-daphnia2/DEB-IBM), we parameterize a model for Daphnia magna with individual level data and validate the model under control and exposure conditions at the population level. We then use this to predict the sensitivity of populations to reductions in various measures of individual performance (feeding, maintenance costs, reproduction, and survival) due to hypothetical chemical stressors in dynamic environments.

RA01-2
Exploring the consequences of spatial heterogeneity in pesticide exposure using an individual-based model of collembolan populations
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Sustainable habitat for soil organisms may be scarce, thus leading to locally high population densities, because soil, being more static than water or air, is heterogeneous: physical conditions often vary widely on a scale of a few centimeters. Moreover, toxic chemicals are likely to be unevenly distributed in the soil as well. To explore the consequences of these heterogeneities for the population dynamics of soil invertebrates, we developed a spatially explicit individual-based model of the collembolan Folsomia candida. In the model, individuals are assumed to sense and avoid contaminated habitat with a certain probability, which depends on contamination level: avoidance of toxicant reduces feeding more harmful to a population than a 20% reduction in reproduction. In part, these questions have been addressed via "elasticity analyses" using mathematical methods. In elasticity analysis, the sensitivity of population growth rate to changes in different generalized modes of action (reduction in survival, reproduction, or growth) is calculated. While these provide a useful estimate of relative sensitivity of one important aspect of populations, intrinsic growth rate alone does not define the dynamics of populations. Most population dynamics are driven in some way via an interaction with their prey species, and a change in the dynamics of individuals may alter this interaction in a way not described by the population growth rate.

The model has been run with two simulation scenarios where polluted area and concentrations of toxicant (CuSO4) are the same, but spatial arrangement and connections between clear patches are different. In both cases the presence of patches of suitable habitat allows the population to grow, although the size is reduced with respect to the control. At the lowest concentration, the organisms cannot sense the toxicant and therefore do not avoid the contaminated areas: for this reason toxic effects are much less pronounced for how environmental conditions (food, temperature, toxicants) are translated into individual performance (growth, reproduction). DEB theory is generic, as differences between species are described via different parameter values within the same model framework. Using DEB-IBM (http://cream-inn.eu/projects/wp-1-daphnia2/DEB-IBM), we parameterize a model for Daphnia magna with individual level data and validate the model under control and exposure conditions at the population level. We then use this to predict the sensitivity of populations to reductions in various measures of individual performance (feeding, maintenance costs, reproduction, and survival) due to hypothetical chemical stressors in dynamic environments.

RA01-3
A comparison of deterministic and stochastic matrix population models to evaluate ecological risk of chemicals
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Environmental management decisions regarding toxic chemicals are mainly based on measurements on individuals, e.g., mortality and fertility. However, the aim of environmental management is, most often, to protect populations, not individuals. The necessary extrapolation from individuals to populations introduces a large degree of uncertainty in the risk assessment process. Presently, this uncertainty is dealt with by using fixed safety factors that are set to be protective in the majority of the cases. One method to reduce this uncertainty may be to use population models. However, before such models can be used routinely in ecological risk assessment of chemicals, the types of models to use must be determined as well as the level of model complexity necessary to provide an accurate risk assessment for different management scenarios. The objective of the present study was to determine whether simple deterministic matrix population models can be used as a first tier in risk assessment of a chemical, or if more complex models that include environmental stochasticity are needed. Matrix population models for two species of fish (eelgou and perch) were used in combination with dose-response data for a metal mixture, 2,3,7,8-tetrachlorodibenzo-p-dioxin, and tributylin. From the deterministic models, the maximum acceptable exposure was determined as the level where the population growth rate (λ) is equal to one, which means that the population size is stable. For the stochastic model, the 5th percentile for λ=1 was used, which means that there is 5% probability for population decline. The results were compared to traditional risk ratios, based on individual level responses, and safety factors (in accordance with European Union guidance documents). Although the results of the stochastic models are more informative with regards to probability for adverse ecological effects, the results of the deterministic models clearly reduced uncertainty compared to the traditional risk ratios. This means that simple deterministic models, in combination with appropriate safety factors, can reduce the risk for over- as well as underprotective risk assessments.

RA01-4
Stochastic density dependent matrix model to extrapolate effects of toxicants from laboratory tests to population-level effects: case study on Folsomia candida exposed to cadmium
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Stochastic density dependent matrix model to extrapolate effects of toxicants from laboratory tests to population-level effects: case study on Folsomia candida exposed to cadmium

SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
This ecosystem model was developed with a focus on EE2-endpoints reliable for risk assessment and will be validated with data from other whole-ecosystem studies performed at Université Laval, modelEAU, Québec, Canada by endocrine disrupters. The objective is to find a compromise between the data available and the complexity required for the model. The ecosystem model is developed with application in ecological risk assessment. This study aims at providing risk managers with an ecosystem model able to predict critical changes in aquatic environments impacted in their decisions. Ecosystem models are required for assessing effects at high levels of organization, but the need for extensive calibration for a specific ecosystem limits their characterizing the ecological impact of such disturbances are costly and time-consuming. Therefore, ecological models are currently being developed to support risk assessors. Endocrine disruption has been measured in many aquatic environments across the world but the consequences on the whole ecosystem are still unclear. Experimental approaches to toxicant-exposed populations. Whether density dependence aggravates or counterbalances effects of toxicants on populations may depend on whether 1) or 1).

RA01-5

Applying an AQUATOX Aquatic Ecosystem Model with FOCUS exposure scenario to assess pesticide effects V. Preziosi1, R.A. Pastorko2, Y. Bramley1, E. Farrell1, D. Huggett1, P. Thorbek1

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Aquatic ecosystem modeling can complement empirical approaches to pesticide risk assessment by allowing efficient evaluation of indirect effects mediated through food-web interactions, testing of multiple exposure scenarios, and assessment of potential long-term effects and recovery times. In a companion poster presentation, we report on the development and calibration of an aquatic ecosystem model based in AQUATOX (version 3.0) for evaluating the potential direct and indirect ecological effects of HerbicideX (hypothetical name) on freshwater systems. We report here on the expansion and application of the calibrated model to: 1) assess potential community-level effects of HerbicideX in a generalized stream characteristic of agricultural areas of northern Europe, 2) demonstrate dose-responsiveness of the AQUATOX stream model, and 3) estimate recovery times of the biological community and key species for cases (if any) where HerbicideX causes significant effects.

The R1 Stream Scenario of FOCUS (2001) was used as the basis for the site characterization and parameterization of physical and chemical elements of the stream model. Nutrient loading similar to HerbicideX experimental mesocosms was used. A fish species (three-spined stickleback) and a dragonfly species (common clubtail) were added to the previously calibrated model, which already had phytoplankton, periphyton, zooplankton, benthic and epiphytic macroinvertebrates, and macrophytes. Immigration and emigration (drift) terms were also parameterized. Information on toxicity and environmental fate of HerbicideX was obtained mainly from the Draft Assessment Report developed by the responsible Rapporteur Member State. An annual time series of exposure concentrations for HerbicideX was developed from a TOXSWA fate model based on the R1 Stream Scenario (i.e., a realistic worst-case scenario). Almost all model species (or groups) showed negligible differences (<1 percent) between control and HerbicideX-exposed cases based on the FOCUS (2001) R1 Stream Scenario, including model species groups or populations of phytoplankton, macrophyte, zooplankton, benthic/epiphytic invertebrates, and fish. Overall, the behavior of the mesocosm-mimic and generalized stream models follows an expected pattern for aquatic systems, with model species responding to physical-chemical conditions and trophic interactions in ecologically realistic ways.

RA01-6

Asystem model for risk assessment of aquatic environments impacted by endocrine disrupters L. Clouzet1, M. Paterson2, A. Dupuis2, P. Blanchfield2, M. Rennie2, K. Kidd3, P.A. Varsolrogehim1

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Endocrine disruption has been measured in many aquatic environments across the world but the consequences on the whole ecosystem are still unclear. Experimental approaches for characterizing the ecological impact of such disturbances are costly and time-consuming. Therefore, ecological models are currently being developed to support risk assessors in decision-making. Ecosystem models are required for assessing effects at high levels of organization, but the need for extensive calibration for a specific ecosystem limits their application in ecological risk assessment. This study aims at providing risk managers with an ecosystem model able to predict critical changes in aquatic environments impacted by endocrine disrupters. The objective is to find a compromise between the data available and the complexity required for the model. The ecosystem model is developed with field data obtained from a multi-year whole-ecosystem study performed at the Experimental Lake Area (ELA, Ontario, Canada): (i) a two years of reference data (ii) three years of exposure to environmentally-relevant concentrations of the synthetic hormone 17α-ethynylestradiol (EE2) and (iii) five years of recovery data. EE2 was chosen because it is one of the most widespread and potent endocrine disrupters. Indeed, the fathead minnow population collapsed after the second year of EE2 additions and endocrine disruption was observed in the other fish species as well. The developed ecosystem model considers direct effects of EE2 on fish species but also the consequences on the whole ecosystem through ecological interactions i.e. feeding and competition relationships. The model is based on simplified equations of the AQUATOX model and chemical elements of the stream model.

RA02 - Approaches for comparative hazard and risk assessment of chemicals

RA02-1

PRIME - an approach and web-based tool for the comparative risk assessment of pesticides P. Mineau1, S.E. Kegley2, M. Guzy3, C. Benbrook1, P.C. Jepson3, K. Benbrook1, T.A. Green1, W.D. Prosnitzhine1, A. Bhat1, L. Presley1, J. Kaplan1

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PRIME is a web-based tool developed by the USDA, Economic Research Service, and U.S. Department of Agriculture in collaboration with other governmental and non-governmental agencies. The tool is designed to estimate the risk of pesticide exposure to humans and wildlife through the food chain. PRIME uses a combination of exposure and toxicity data to estimate the risk of pesticide exposure to humans and wildlife through the food chain. The tool is designed to estimate the risk of pesticide exposure to humans and wildlife through the food chain. The tool is designed to estimate the risk of pesticide exposure to humans and wildlife through the food chain. The tool is designed to estimate the risk of pesticide exposure to humans and wildlife through the food chain.
provides to organize these criterias systematically and conclude priority numbers for the risk management activities. Risk magnitude (RM) can be inferred by assessing expert opinions instead of strict formulations. Fuzzy inference (FI) systems are used for complex matrices that cannot be regulated or formulated. It is aimed to propose an approach for ERA of pharmaceuticals based on FAHP and FI system as an objective and sensitive method in order to overcome uncertainties and compensate data gap in literature. A separate hierarchy was developed for RL, RS and RP in order to get the scores of AHP for inference of RM. Characteristics of the pharmaceuticals and environment and environment evaluation were considered as the subfactors of the RL in order to assess fate/transport of the pharmaceutical in the environment and possible exposure pathways for the ecosystem. Effect assessment is conducted by using RS hierarchy including subfactors of ecotoxicological effects of pharmaceuticals and environmental characteristics that contribute the magnitude of these effects. RL hierarchy contains the sources of the pharmaceuticals and factors related with treatability indicating the possibility of release to environment. On the other hand, RS and RP factors were identified based on expert opinions and RM for the case evaluated is achieved. Risk assessment of pharmaceuticals in the environment by using AHP procedure to consider all of the factors contribute to risk related both with pharmaceuticals and environment in terms of risk components that are RL, RS and RP. FI method enables to use expert opinions instead of ampic formulations which is beneficial for the complex assessments that are affected by numerous factors and cannot be completely formulated like ERA. Proposed approach reduces uncertainties and subjectivities and provides more rigid RM that can be used as a guide for the risk management attributes.

RA03-2 Exposure hazard assessment: lessons learned from cyclic volatile methyl siloxanes
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Two cyclic volatile methyl siloxanes, octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), have recently been subjected to close scrutiny of their exposure hazard by the European Union. These chemicals have unusual physical chemical properties which place them outside of the domain of well-studied organic chemicals. It is thus not surprising that difficulties were encountered when existing methodologies for exposure hazard assessment were applied to these chemicals. Here we explore these difficulties with the aim of extracting the lessons that can be learned from these chemicals.

RA03-3 A new methodology for PBT prioritization of chemical inventories
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A new PBT prioritization methodology has been developed in view of regulatory requirements for quantitative assessment of persistence, bioaccumulation potential and toxicity of chemicals. CATALOGOC/OASIS and EPI Suite QSAR models for calculating P, B and T endpoints have been used. The methodology is implemented in a software system for computerized PBT classification of large chemical inventories. The developed methodology introduces fate/hazard classification of chemicals based on regulatory threshold values for identifying PBT substances. Thresholds corresponding to the variation of respective test procedure results are further introduced defining uncertainty bright lines around the central threshold values. The model applicability domain of each used QSAR model is incorporated in the scheme in order to evaluate confidence of obtained estimates. The developed approach introduces a two-stage PBT classification scheme - primary classification of parent chemicals and secondary classification of stable degradants based on regulatory threshold values for identifying PBT substances. Thresholds corresponding to the variation of respective test procedure results are further introduced defining uncertainty bright lines around the central threshold values. The model applicability domain of each used QSAR model is incorporated in the scheme in order to evaluate confidence of obtained estimates. The introduced secondary classification based on stable degradants provides an in-depth PBT profile. Following a more conservative approach of applying the prioritization scheme, identification of a hazardous stable degradable is considered grounds for inclusion of the parent chemical in a higher priority class.

RA05-2 Comparative risk assessment of arsenic trioxide and its substitutes for occupational exposure in Murano (Venice, Italy) artistic glass production
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Arsenic trioxide is included in the Candidate List of Substances of Very High Concern (SVHC) and soon it could be subject to authorization under the REACH regulation, because of its properties of carcinogenicity, mutagenicity and toxicity for reproduction (CMR substance). In the district of Murano artistic glass (Venice, Italy), arsenic trioxide has being used since centuries as additive for refining and bleaching melting glass. It is estimated that about 8 tons per year of arsenic trioxide are currently used in Murano glassworks. Small particles of arsenic powder can be easily inhaled by workers; furthermore, when melting the glass mixture at high temperatures, arsenic toxic fumes are released. To reduce the occupational health risks of glass production, the use of suitable substitutes for replacing arsenic trioxide is being explored and cerium oxide and blast furnace slag are among the most promising candidates. A comparative study has been performed with the aim of evaluating the occupational health risks of substitutes substances in comparison with arsenic trioxide. Specifically, the aim of this study is: i. to assess arsenic trioxide health hazard in comparison with cerium oxide and blast furnace slag; ii. to develop for all production phases suitable exposure scenarios for workers, representative of a typical glassworks; iii. to quantify inhalation and dermal contact exposure to arsenic trioxide and its substitutes through the application of different occupational exposure models (i.e., ECETOC worker tool, MEASE and ART) and to compare models’ performance for inorganic substances in the selected exposure scenarios; iv. to estimate and compare health risks for glass workers associated to inhalation and dermal contact for the substances of interest. The comparative risk assessment procedure proves to be effective in demonstrating that the use of cerium oxide and blast furnace slag would reduce the health risks associated to occupational exposure. Cerium oxide and blast furnace slag are therefore confirmed as interesting candidates to replace arsenic trioxide in artistic glass production.

RA06-2 Comparative hazard and risk assessment of flame retardants (Prototypical Case Study): ENFIRO
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Several brominated flame retardants (BFRs) have unintended negative effects on the environment and human health. Some of them show a strong bioaccumulation in aquatic and terrestrial food chains, some very persistent and show some serious toxicological effects such as endocrine disruption. During the last decade, an increasing number of reports have presented evidence of these negative effects caused by BFRs. A number of BFRs (in particular polychlorinated diphenyl ethers (PBDE’s), hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBP-A)) can be found in increasing concentrations in the human food chain, human tissues and breast milk (1,4,7,10,13,16-Hexabromocyclododecane (HBCD)). These BFRs are not only found in aquatic and terrestrial environments, but also in human tissues and breast milk around the world. These observations suggest that BFRs are not just new contaminants, but that they have already entered the human food chain. This paper presents an overview of the current knowledge about the occurrence, fate and health effects of BFRs in the environment. It provides an overview of the different regulatory frameworks that have been or are being developed to control the use of BFRs. The paper also discusses the challenges and opportunities for future research on BFRs.

RA03 - Are Environmental Specimen Banks ready to face future challenges of environmental chemistry and regulatory toxicology?
RA03-1 A fish tissue archive for monitoring chemical pollution in UK rivers. How it operates and its application to EU priority substances
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The UK Fish Tissue Archive
In 2007 CEH and the Environment Agency in England and Wales (EA) began to build a fish tissue archive by collecting 10 fish (generally roach) per year from a number of river sites and storing them long term at -80°C to provide a resource for future prospective monitoring of pollutants. Measuring pollutants in biota is often preferable to water because fish and other biota accumulate chemicals both from the water and the food chain over their lifetime,
Environmental Quality Standards (EQS)

EU Directive 2008/105/EC lists freshwater EQS for over 30 substances, but it includes only an (optional) biota standard for 3 (article 3, 2a) requiring member states that use this characteristic and its contents, an EQS of 20 μg/kg, and/or for hexachlorobenzene, an EQS of 10 μg/kg, and/or for hexachlorobutadiene, an EQS of 55 μg/kg, these EQS being for prey tissue (wet weight), choosing the most appropriate indicator from among fish, molluscs, crustaceans and other biota.

**Results**

We analysed some of the fish from the UK Fish Tissue Archive for these three substances and found:

**Mercury** which was unambiguously detected in marine and freshwater species and is still released as a trace component of fossil fuels and some electrical components was analysed in roach. About half the samples exceeded the EU EQS of 20 μg/kg (median: 23 μg/kg), but even the maximum of 68 μg/kg is about a factor of 10 below the EU food limit of 500 or 1000 μg/kg depending on fish species.

**Hexachlorobenzene**, a fungicide which is no longer used in the EU, was below the EQS of 10 μg/kg in all fish analysed. Eels had higher concentrations with a median of 1.8 and a maximum of 6.4 μg/kg compared to 0.9 and 4.5 μg/kg in roach.

**Hexachlorobutadiene** which was in the past used as a solvent in production and as a fungicide and seed dressing and is still generated in small amounts as a by-product, was not detected in the majority of samples.

**Conclusions**

- The samples stored in the Fish Tissue Archive are well suited for monitoring of priority substances, especially those for which an EQS has already been set.
- Mercury levels are some concern in English rivers.
- As the Fish Archive grows it will be possible to determine temporal and spatial trends of these and other substances.

**RAO-3.2**

**Environmental monitoring data: support for an effectiveness assessment and a success control under REACH**

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Environmental specimen banks support competent authorities in the assessment of chemicals. Together with national and EU-wide monitoring systems they can give information for the identification of new substances of regulatory concern. Beyond the assessment of individual chemicals, monitoring data can also be used to develop indicators to evaluate the implementation of different chemical regulations by providing information on the impact of chemicals to human health and different compartments of the environment.

By 2012, the European Commission has to present a first evaluation of the effectiveness of the REACH regulation. Human biomonitoring and environmental monitoring programmes may provide valuable data to underpin this evaluation. In the EU there is a variety of environmental monitoring activities which have been established for various reasons. They are - in different degrees - suitable to indicate the implementation of REACH. A research project - funded by the German Environment Protection Agency (UBA) - analysed the available monitoring programs (including environmental specimen banks) to identify those who can contribute to evaluate the effectiveness and success of different REACH task, starting from specific tasks related to specific parts of REACH (e.g. registration) up to tasks related to the effectiveness evaluation of REACH as a whole.

In order to determine appropriate indicators and methods to evaluate the impact of the European chemicals legislation REACH, a detailed and complete review on ongoing environmental monitoring activities has been conducted. Furthermore, a guidance document for the use of environmental monitoring data with a view to the evaluation of chemicals has been developed and presented. It includes both the company’s self-monitoring as well as government monitoring and important regulatory focal points under REACH. Exemplary case sheets on selected chemicals were documented for the monitoring / identification of substances of concern, thereby using the Environmental Specimen Bank (ESB).

Future challenges in regulatory risk assessment go beyond time trends of individual substances. Effectiveness assessments of such REACH require indicators for the total environmental burden due to the wide dispersive use of a large number of substances - and its effect on biodiversity. Environmental specimen banks can support such assessments and the elaboration of adequate indicators.

**RAO-3.3**

**Mercury stable isotopes in seabird eggs from the NIST Marine Environmental Specimen Bank reflect a gradient from terrestrial geogenic to oceanic mercury reservoirs**

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Mercury stable isotopes in murre eggs from the NIST Marine Environmental Specimen Bank for reflect a gradient from terrestrial geogenic to oceanic mercury reservoirs. A total of 420 samples collected from the period of 1981-2009 and from two cities in Germany: Münster (n = 10 samples (5 male and 5 female) per year from 1981-2009) and Berlin (n = 10 samples (5 male and 5 female) per year from 1995-2009, for a total of 150 samples), were analysed in roach for these three substances and found: About half the samples exceeded the EU EQS of 20 μg/kg (median: 23 μg/kg), but even the maximum of 68 μg/kg is about a factor of 10 below the EU food limit of 500 or 1000 μg/kg depending on fish species.

**Conclusions**

- The samples stored in the Fish Tissue Archive are well suited for monitoring of priority substances, especially those for which an EQS has already been set.
- Mercury levels are some concern in English rivers.
- As the Fish Archive grows it will be possible to determine temporal and spatial trends of these and other substances.

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**RAO-3.4**

**Temporal trends in dioxins and dl-PCBs from Baltic herring (Clupea harengus)**

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The unintentional release of dioxins (dibenzo-p-dioxin (PCDD), dibenzofuran (PCDF), and dioxin like PCBs (dl-PCB)) into the environment was at its peak prior to the 1980s [1]. Since then, extensive measures have been taken to reduce dioxin emissions within the EU e.g., the Helsinki Convention (1974, 1992), the Stockholm Convention on Persistent Organic Pollutants (POPs), the plan for integrated pollution prevention and control (IPPC) [3]. However, dioxin concentrations continue to be higher than expected in BalticReference 4 fish. A large proportion of fish caught in the Baltic Sea exceed the limit for marketing fish within the EU [11] (4 pg WHO1997 TEQ/kg wet wt (WHO1997 TEQ/kg wet wt) and 8 pg WHO1997 TEQ/kg wet wt (WHO1997 TEQ/kg wet wt) (WHO/chlorinated dioxins) WHO1997 TEQ/kg wet wt) (WHO/chlorinated dioxins). In order to determine appropriate indicators and methods to evaluate the impact of the European chemicals legislation REACH, a detailed and complete review on ongoing environmental monitoring activities has been conducted. Furthermore, a guidance document for the use of environmental monitoring data with a view to the evaluation of chemicals has been developed and presented. It includes both the company’s self-monitoring as well as government monitoring and important regulatory focal points under REACH. Exemplary case sheets on selected chemicals were documented for the monitoring / identification of substances of concern, thereby using the Environmental Specimen Bank (ESB).

Future challenges in regulatory risk assessment go beyond time trends of individual substances. Effectiveness assessments of such REACH require indicators for the total environmental burden due to the wide dispersive use of a large number of substances - and its effect on biodiversity. Environmental specimen banks can support such assessments and the elaboration of adequate indicators.

**RAO-3.5**

**Temporal trends in human exposure to fluorinated chemicals in relation to changes in the surrounding environmental conditions**

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A total of 420 samples collected from the period of 1981-2009 and from two cities in Germany: Münster (n = 10 samples (5 male and 5 female) per year from 1981-2009 (no sample for 1994), for a total of 270 samples) and Halle (n = 10 samples (5 male and 5 female) per year from 1995-2009, for a total of 150 samples), were analysed for a suite of fluorinated analytes. The specific targets include current and legacy commercial fluorinated surfactants, such as the polyfluoroalkyl phosphoric acid diesters (dPAPs), the N-ethylerufluorooctanesulfonamidoethanol-based polyfluoroalkyl phosphoric acid diesters (dPAPs), and the perfluorooalkyl acids (PFAAs). Both the dPAPs and various PFAAs, that include PFOA and PFOS, have been detected in human blood. Among the 420 samples analyzed here, the perfluorosulfonates (PFSAs: i.e., C6 (0.053 - 3.81 ng/g)
and C8 (0.318 - 79.0 ng/g)), perfluorooctanesulfonamidoacetates (N-MeFOSAA (<0.0031 - 8.11 ng/g), N-EtFOSAA (0.0058 - 9.00 ng/g), FOSAA (<0.0011 - 8.25 ng/g)), and C8 (0.176 - 31.7 ng/g), C9 (0.020 - 2.70 ng/g), C10 (0.020 - 0.880 ng/g), C11 (0.003 - 0.555 ng/g) were detected in over 80% of the samples, and C8 (0.020 - 2.70 ng/g) and C9 (0.0001 - 2.71 ng/g) were observed in 50% of the samples.

**RA04-3** Life-cycle Assessment of biofuels for transportation: understanding the effects of scale

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Life-cycle assessment of biofuel production and use projects have yet been done on including eutrophication. The aim of LC-IA is to develop a functional model and spatial and temporal differentiated model, as both central fate processes, sensitivities of receiving environments (e.g. differences in limiting nutrient and variations in this over the year) and the resulting damage can show important spatial variations. Both the midpoint and endpoint (damage) modelling are to be included and the aim is to base the damage modeling on dose-response curves expressing the correlation between the (increase in) nutrient concentration and the potentially affected fraction of species in the marine ecosystem. This paper presents the first draft on the midpoint model for global marine eutrophication due to nutrient emissions for the emission sources “fertilizer in agriculture”, “manure in agriculture”, “N-fixation in agriculture”, “air emissions of NH3”, air emissions of NOx and “water emissions” including direct sewage water emissions and emissions after sewage treatment.

**RA04-4** Life-cycle Assessment of biofuels for transportation: understanding the effects of scale

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1University of California, Berkeley, Berkeley, United States of America

Life-cycle assessment is used as the framework to study the effects of scale throughout the entire life-cycles of biofuels. Preliminary results indicate that growing biomass for fuel at an industrial scale presents a number of unique challenges as well as opportunities. We show that economies of scale make a decisive difference between industrial-scale and lower-scale (“boutique”) biofuels. For the United States, we show that making 40 billion liters of biofuels by a deadline only one year away is an economically feasible environmental improvement. 2. Simplistic present scenarios for land conversion, trying to answer the question where and on what kind of land will biomass of this scale be grown. We quantify soil organic carbon emissions from massive biomass cultivation, and the need of crops for water, fertilizer and biocides. We assess the greenhouse gas, water consumption, and criteria air emissions of converting cellulose biomass into ethanol at industrial scale.
The results are for the year 2008. The expected results are: an aggregated measure of the toxicity from the sources included in E-PRTR, a calculation on contribution to toxicity by different sources; and information on each substance’s contribution to toxicity, identification of the most important sources. For these four the results are divided in contribution to human toxicity and to ecotoxicity. This study makes it possible to discuss how useful this method is to quantify the chemical footprint by using data from E-PRTR and the USEtox method.

RA04B-1
Probabilistic environmental hazard assessment of implementing green chemistry property design guidelines to reduce acute and chronic aquatic toxicity
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One of the Twelve Principles of Green Chemistry emphasizes the need to synthesize safer chemicals. These chemicals should be capable of performing their desired function but would be designed to elicit minimal toxicity. In support of this effort, two recent studies by Youchov et al have explored the relationships between chemical properties and acute or chronic toxicity as measured through standardized OECD and EPA protocols. Mechanistically-rationalized guidelines were derived resulting in two practical design guidelines: acute and chronic toxicity could be minimized if compounds had an octanol-water partition coefficient (log Pow) below 2 and HOMO-LUMO gap (dE) greater than 9 eV. In this study, we examined the potential utility and effectiveness of these design guidelines to reduce acute and chronic aquatic toxicity of common industrial chemicals using probabilistic environmental hazard assessment (PEHA). Chemical Toxicity Distributions (CTDs), a type of PEHA modelling approach, are ideal for predicting the probability of encountering industrial chemicals exceeding established US EPA thresholds of standardized acute and chronic toxicity to algae, cladoceran and fish models; 2. to predict the likelihood of exceeding these thresholds if chemical safety guidelines were followed; and 3. to examine acute and chronically toxic chemicals, chemical classes and modes of action of chemicals that may not be “designed out” by chemical property guidelines. For example, in the absence of chemical design guidelines, our model predicts that 14.5% of chemicals would be classified by the EPA as being of “High Level of Concern” for acute toxicity (LC50 of 0-1 mg/L) to the fathead minnow. However, if log Pow and dE guidelines were employed during chemical development, only 3.3% of industrial chemicals are predicted to be of High acute toxicity concern to the fathead minnow. If the two green chemistry design guidelines put forth by Youchov et al are employed, the present study predicts that reduced acute toxicity to the fathead minnow could be achieved for over 10% for industrial chemicals classified with High acute toxicity.

RA04B-2
Biodiversity footprinting - quo vadis?
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Biodiversity loss, and its potential impacts on human welfare, are an increasing environmental concern, and should be assessed in product assessments, in order to provide
adequate support to decision-makers. Likewise, there is an upcoming trend to quantify environmental impacts of products in terms of so-called footprints, which should address biodiversity effects in addition to the conventional carbon and water footprints. However, biodiversity is a multifaceted concept with strong variation in time and space, which makes it difficult to assess. Some current methods are based on the unit “potentially disappeared fraction of species” (PDF), but the scale (e.g. biodiversity loss at the regional or global level) and choice of which species should be protected are not consistently defined across impact categories. Therefore, impacts to biodiversity at local, regional or global scales are often aggregated, leading to misinterpretations of LCA results. Here, we attempt to provide a framework to guide methodological choice. Clarifying why we are concerned about biodiversity loss is the first step towards determining what we should be measuring. We distinguish between valuing biodiversity due to intrinsic value or due to its utility for mankind. For the first, enough primary habitat needs to be conserved and the human-modified landscapes need to be managed to avoid global extinction of species. The utility can be manifold and the concept of ecosystem services could serve as a starting point that helps to structure, classify, and measure different functions that ecosystems provide for humans. We also highlight value judgments involved in assessment frameworks, indicators and weighing schemes. To assess cause-effect chains of biodiversity loss, multivariate models, meta-analysis of literature of cause-effects from field surveys, extrapolation of lab experiments or expert opinion can be used.

RA04B-3 Valuating ecosystem goods and services in LCA
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In LCA models, resource assessment is centered on the notion of scarcity: depletion is evaluated using a static perspective by comparing a resource’s rate of abstractions versus the available stock and its rate of regeneration, or by quantifying the marginal extraction cost. As a result, a limited number of resources (essentially fossil fuels and minerals) in industrial production goods and services (EGS) are basically ignored, despite their crucial role. Dynamic accounting is a prerequisite to consistently embrace the actual pressure of human activity on renewable resources, their marginal rate of regeneration and the independency of their production patterns. Hence, ecological modeling can be a way to build a consistent and practical framework of characterization of EGS in LCA. The GUMBO model details the internal behaviour of the global natural system (geobiosphere) and its interactions with the anthroposphere; it is adjusted with key metrics measured during the 20th century to reflect global dynamics. The model delivers the economic values of human, social, built and natural capitals (i.e. EGS) according to user-defined scenarios for the societal mankind during the 21st century. The use of monetary units enables direct comparisons across ‘capitals’ and is a strong vector to communicating results. However, it drives to a utilitarian, end-user perception of EGS’ usefulness, disregarding the role of indirect EGS and the past natural ‘investment’ to this capital. Therefore, other metrics developed in the field of sustainability indicators may be selected, such as exergy. Acknowledging the exergy fruitful provides a measure of the global-scale geobiosphere work with a common physical numeraire (equivalent Joules of solar energy, seJ). We expect GUMBO to improve consistency in the exergy evaluation of global natural processes and resulting Unit Emergy Values (UEVs) of EGS, which can be used to obtain complementary (physical) information to the (economic) characterization of EGSs in LCA. Although regionalization of this framework remains unclear, it is expected to be highly representative at global scale and open the road to a comprehensive, ecology-oriented perspective in the evaluation of the natural capital and its use by humans. The aim of this presentation is to illustrate the methodological approach and its preliminary outcomes.

RA04B-4 Exergetic footprint as indicator to assess the environmental sustainability of processes
E. Roca, A. Saravia, M. Herva, C. García-Díez
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Exergy analysis has two key attributes for being used as an environmental indicator: first, given that the environment is used as a reference state, exergy is a measure of any thermodynamic deviation with respect to its normal state; second, it allows comparisons between inflows and outflows, regardless they are mass or energy streams, using the same physical units (exergy) for their analysis. Besides, the exergetic footprint (ExF) bears in mind the exergy that the still remains in products and highlights the use of exergy as a convenient unit of measure and comparison. In this paper, the (ExF) was used as a resource/waste accounting indicator for the environmental assessment of a production process. The proposed methodology was applied to a particleboard production process from a factory located in Galicia (NW Spain). The study was proposed as a gate to a production chain. The incoming streams were categorised as raw materials, secondary materials and energy sources, while the output streams were the generated waste and the produced particleboard. 1 m3 of produced particleboard was selected as functional unit. Raw material for the production process was wood from the local (regional or global level) and choice of the recycled wood used until the complete substitution of the raw material on the ExF, some scenarios of the production process were built considering the increase ratio of recycled wood used reaches a value of 17,05 GJ per functional unit. Meanwhile, to determine the ExF the exergy that still remains in the produced particleboard was also considered, and a value of 3.5 GJ per functional unit was obtained. For the scenarios built, CEx ranged from 17.76 to 15.73 GJ m-3 particleboard and the ExF from 3.69 to 1.66 GJ m-3, for 0 to 100% of recycled wood wastes employed. The material resources, especially wood material, were the main contributors to ExF and CeF, as it had been identified by the ecological footprint in a previous work. The results suggest that a clear improvement in the sustainability of the process could be attained by increasing the feasibility of using different waste materials in the design of the particleboard.

RA04B-5 Thermodynamic resource indicators and footprint in LCA: a case study of titania in China
W. Liao, R. Heijungs, G. Huppes
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LCA has standardized methods for assessing emission impacts but no comparable methods for the accounting or impact assessment of resource use. This study contributes to the existing research by offering a comparative comparison of different resource indicators, in particular those based on thermodynamics, and testing them in a case study of titania in China. The system boundary for resource indicators and the case study is defined as a thermodynamic hierarchy at four levels. Seven resource indicators are applied. Four are thermodynamic: cumulative solar energy demand (CED), cumulative energy demand (CExD), and cumulative exergy extraction from the natural environment (CEENE), and three have different backgrounds: abiotic resource depletion potential (ADP), environmental priority strategies (EPS), and eco-indicator 99 (EIP99). The foreground Inventory data has been collected through on-site interviews and visits. The background inventory data are from the database ecoinvent v2.2. Resource indicators are based on CML-IA database. Computations are with the CMLCA software. Results show that resource indicators such as CED, CExD, and CEENE have similar scores while their five orders of magnitudes lower than the CED score. Atmospheric thermodynamics do not contribute to the CED or CEEND score. Land resources account for a negligible percentage to the CED score and a small percentage to the CEENE score. Non-renewable resources have a dominant contribution to all resource indicators. The global production of titania would account for 0.12% and 0.14% of the total anthropogenic non-renewable resource demand in terms of energy and exergy, respectively. The entropy footprint of global titania is 5.2 E-07 m2. We demonstrate the feasibility of thermodynamic resource indicators in Chinese industries. To the extent that the resource indicators to take different resource issue as their key problem and have higher environmental relevance in terms of expressing the resource scarcity and depletion than the thermodynamic ones. As the conceptual basis for the several indicators differs fundamentally, different cases might well show more diverging outcomes.

RA04B-6 European guidelines for measuring the environmental footprint of products and organisations
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In close co-operation, DG Environment and the Joint Research Centre of the European Commission are developing European guidelines for measuring the Environmental Footprint of products (covering goods and services) and organisations in the EU (NW Spain). The objective is to support decision-making processes in business and policy with a comprehensive multi-criteria set of indicators based on the robust and quality assured measurement of environmental performance. The guidelines provide guidance on how to calculate a Product Environmental Footprint (PEF) and an Organisation Environmental Footprint (OEF), as well as how to create product category or sector specific requirements for use in Product Footprint Category Rules (PFCRs) or Organisation Footprint Sectoral Rules (OFSRs). Each requirement in the PEF and OEF guidelines has been taken into consideration the recommendations of existing environmental accounting methods. Although existing methods align on some of the guidance they provide, discrepancies and/or lack of clarity remain on a number of important decision points, which significantly reduces the consistency and comparability of the results. In some cases, it was necessary to go beyond the level of guidance provided in existing documents. An example is the coverage of impact categories and the models used for calculation.
Transformation/dissolution of copper alloys: comparison of two anti-abrasion devices for massive sample testing

Transformation/Dissolution (TD) test (2001). The T/D results of samples embedded in epoxy resin showed lower experimental noise compared to those for the polypropylene wheels and is therefore considered as a more appropriate methodology for transformation/dissolution testing of massive forms of copper.

Microscopy studies of samples surface concluded that defects in the alloy surface during the wire production were responsible for the higher variability in the T/D results of the polypolyphenol wheels protocol, compared to the epoxy polished sample protocol.

The surface-specific metal releases from the alloy samples, using both anti-abrasion protocols, usually increase linearly with time, with faster dissolution rates for lead and zinc and lower rates for copper and nickel. The dissolution kinetics of lead in three samples, demonstrate a decrease in the metal release ratio as the tests progress, suggesting a depletion of lead from the alloy surface. For copper, the main component of the alloys, the dissolved copper concentrations measured in the alloy were between 0.04 and 2.05 mg/L, compared with the values observed from pure copper samples (0.14 to 0.41 mg/L).

Transformation/dissolution of copper concentrates: effect of mineral composition on metal solubility

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Metal release rates were observed for copper and arsenic (0.2-4%) and the highest release rates were observed for lead (7-35%). The detailed kinetics from the T/D tests show that the metal release rates generally decrease as a function of time. Copper releases from copper minerals are lower for Chalcocite (0.3% copper release during 7 days TD) and highest for Chalcocite (5% copper release during 7 days TD). From the data, it can be concluded that it will not be possible to derive one release or one environmental classification entry applicable to all copper concentrates. Comparison of release data among concentrates and their mineralogy clearly indicate that the metal release rates depend on the elemental and mineral composition of the copper concentrate. The results further indicate that, as demonstrated for copper, the metal releases from copper concentrates can be predicted from the mineral composition of the copper concentrate. The results further indicate that, as demonstrated for copper, the metal releases from copper concentrates can be predicted from the mineral composition of the copper concentrate. The results further indicate that, as demonstrated for copper, the metal releases from copper concentrates can be predicted from the mineral composition of the copper concentrate. The results further indicate that, as demonstrated for copper, the metal releases from copper concentrates can be predicted from the mineral composition of the copper concentrate.
A unit world model for metals in lakes, TICKET-UWM, has been developed that considers key processes affecting metal transport, fate, and toxicity including complexation by aqueous inorganic and organic ligands (e.g., DOC), adsorption to particulate organic carbon (POC), binding to biological receptors (biotic ligands), and transport of dissolved metals and solids between the water column and sediment. The TICKET-UWM was used to assess the rate at which metals (Cu, Pb, Zn, Ni, Co, and Cd) are removed from the water column in a generalized lake system through partitioning and settling. The model was also used to assess metal speciation changes in the sediment and the potential for metal remobilization from sediment.

Model results indicate that, in most cases tested, greater than 70% of the metal added to the water column was removed within 28 days. Results also suggest the potential for remobilization of metals is limited, particularly when acid volatile sulfide (AVS) is present to precipitate metals as metal sulfides.

RA06-5
A BLM probabilistic approach to integrate variability in the derivation of Water Quality Criteria at regional and local scales
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The biotic ligand model (BLM) is one of the more promising models allowing to derive Water Quality Criteria (WQC) based on true bioavailable fractions of metals. However, several physico-chemical parameters must be assigned for running BLMs (i.e. pH, major cations, DOC) and the operational use of BLMs can suffer several flaws because of:
(i) the lack of comprehensive data.
(ii) natural variability. Rivers and lakes can indeed show great spatial and temporal variations. So far however, only single values were generally considered for describing variables of concern, and the high natural variability with time and along a river watershed was ignored.

The main objective of this study was then to investigate the potential of probabilistic approaches able to capture the uncertainty of variables of concern and the incompleteness of datasets for BLMs application on large scales. The methodology was tested on the Loire river watershed for copper, where different spatial scenarios were considered. Data availability for copper showed that a significant fraction of dissolved concentrations (about 50%) were below the limit of detection. In order to judge realistic values to these non-detects, 'distributional' methods were used. In addition, probabilistic risk assessment was also conducted at local scale (i.e. considering data collected at a given monitoring station only). In such a case, the number of available data can be reduced dramatically and frequentist statistics can be more subject to criticism. To overcome the problem of data scarcity, a Bayesian approach was proposed.

Results showed that PNEC values calculated with or without probabilistic approaches significantly differ, e.g. if non-detects are ignored, the risk index is above one (situation at risk), while it is below one if a distributional approach is considered (situation at risk). Similarly, in case of scarcity of data at local scale (i.e. at a given monitoring station), frequentist and Bayesian approaches led to results significantly different.

RA06-6
Accounting for both local aquatic community composition and bioavailability in setting local quality standards for metals
A.J. Peters, P.C. Simpson
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Recent years have seen considerable developments in the ability to make water quality standards for trace metals more ecologically relevant by taking account of the effect of local water chemistry conditions on bioavailability. This prevents situations where a different level of risk is considered acceptable at different sites due to changes in bioavailability which are not accounted for in the standard. We describe preliminary efforts to address an additional issue in the development of water quality standards which is specific to particular locations, by taking account of the composition of the local ecological community (the ultimate protection objective). This has occasionally been addressed through the use of field measurements to derive species sensitivity distributions (SSD) in sediments. An alternative approach, which combines a quality assessed ecotoxicity dataset with field measurements of the abundance of benthic macroinvertebrates to derive an SSD on the community which is either expected to be present, in the absence of anthropogenic pressures, or the community which is present at the sites. Site specific standards are derived for zinc in an impacted by historic mining activities. Site-specific targets for zinc, based on the macroinvertebrate ecology predicted or observed at a site, can be derived and can result in improved compliance compared to the traditional and bioavailability-based EQS. In addition, to zinc, the approach is likely to be applicable to other metals and possibly other types of chemical stressors (e.g. pesticides). However, the methodology for deriving site-specific targets requires additional development and validation before they can be robustly applied during surface water classification.

RA06 - Contaminated sediments in a changing environment

RA06-1
Bioavailability and beneficial use as primary demands for a management guidance of contaminated dredged sediments
E. Came, R. Wolfe, A. Scheffeler
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Management of contaminated sediments is a key issue in order to establish a sound development of the Baltic Sea and contributing to the EU Strategy for the Baltic Sea Region (EUSBSR). This issue is of utmost importance according to HELCOM and addressed by the BSR funded project SMOCS (Sustainable Management of Contaminated Sediments). The main delivery of SMOCS comprises a guideline, including tools for assessment of sustainability, and decision support. The purpose of this lecture is to present the draft guideline on sustainable management of contaminated sediments for dredging projects all around the Baltic Sea. This project is a result of the joint EU-project SMOCS under consideration of actual national and international regulations, integrating other projects as well as interests of stakeholders. For example, the participants of a workshop preferred a guidance document giving new ideas and an overview of options for dredged material management. Exact definitions of contamination of dredged material (when to call dredged material "clean", "contaminated" or "hazardous") and action levels were required. Emphasis was given to fulfill political demands as to avoid waste production. Therefore it was primarily recommended to assess the feasibility of beneficial use of the sediments. Bioavailability concept for the derivation of sediment quality standards has a challenge to foster this approach, because not the total amount of contaminants is of importance rather than the biological available part. Such risk assessment based on laboratory or in situ studies meets the request of the European Water Framework Directive to develop a toxicity-based bioavailability model to estimate the risk of sediment-associated contaminants. The purpose of an LCIA probably in combination with a Risk Assessment is to ensure that dredging activities are performed in an environmentally acceptable manner, use sound engineering techniques, which they are economically warranted and take sufficient consideration of long term effects. The choice of appropriate indicators is given in detail as part of a Decision Support Tool.

RA06-2
Natural attenuation in sediments
M. Van Bemmelen, M.I.H.A. Wagelmans
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Due to polluted groundwater in urban areas, sediments in urban water streams become polluted as well. This can cause pollution of surface waters. Natural attenuation of chlorinated hydrocarbons in sediment can be used to sustainably manage or remediate groundwater pollution. Due to the change in remediation procedures in the Netherlands from site specific remediation to area specific remediation, an approach based on natural attenuation will be increasingly considered. Provided that the risks of this approach can be controlled, natural attenuation processes in sediment can be a valuable contribution to area specific management and remediation plans.

However, there are still some knowledge gaps about these natural processes. Also research methods to quantify these processes are lacking. And the possible ecological effects in sediment or surface waters are still unknown.

From the knowledge gaps, research questions have been defined. How are the most important processes related and can these processes be modeled? Where does biodegradation occur? How fast is this process? Can it be stimulated? Which techniques can be used for monitoring?

Six polluted sites in three cities in the Netherlands were sampled, including 1 reference sample per site. Samples were either frozen in liquid nitrogen or the individual sediment layers were sliced and sampled in the field. Chemical and molecular (Q PCR) analyses have been performed in order to characterize the sludge and remediation.

The project results show that in the toplayer of the sediment, which contains the higher organic matter content, anaerobic conditions are present. In this layer the microbial activity is higher than in deeper sediment layers. The dechlorinating capacity in this layer is higher than in deeper layers. However, in the toplayer, not only anaerobic organisms are present, but also aerobic and micro-aerobics. This implies that microaerophilic conditions are present and chlorinated ethenes can be both anaerobically and aerobically be degraded. In the layer below, the dechlorinating capacity is lower. This has implications for dredging management in this specific canal.

Dredging of the canal would, at least temporary, decrease the dechlorinating capacity. This could cause an increase in chlorinated ethenes in surface water and result in ecological risks during a certain period of time. The rest of the results will become available in December and January.
K. L. Moshenberg, S. Heise

Hamburg University of Applied Sciences, Hamburg, Germany

Assessing the impacts of climate change on the fate and transport of HCB and Cd in the Elbe River Basin

K.L. Moshenberg, S. Heise

University of Hamburg, Hamburg, Germany

sediment concentrations of HCB (HCB) and Cadmium (Cd) in sections of the Elbe River basin significantly exceed maximum allowable concentrations established by the European Commission. Even though concentrations of both contaminants have decreased significantly over the past 15 years, levels remain elevated. In addition, previous studies have documented that downstream transport of contaminated sediment occurs primarily during high water events. Given anticipated climate change induced changes in mean discharge, potentially including an increase in the magnitude of high and low water discharge events, this study seeks to evaluate the role of climate change in the long-term fate and the transport of coarser sediments and associated contaminants, a hydrodynamic and cohesive sediment transport model for a 230 km segment of the Middle Elbe River was developed. Of particular interest is contaminant transport to floodplains and retention time in the numerous groyne fields that line the banks of the Elbe River. Multiple climate change scenarios involving changes in the amplitude and phase of yearly streamflow were used to simulate contaminant transport throughout the Elbe River basin at 25 and 50 years in the future. Integration of modeling output with the results of recently conducted (2010, 2011) sediment data enabled an enhanced understanding of current and potential future dynamics of HCB and cadmium transfers in the Elbe River modeling results, challenges simulating transport in groyne fields, techniques for resolving data gaps, and broad-scale suitability of the model to European river basins are discussed.

RA01-1

The gap between scientific publication and the press - a case example

F. Schwab1, 2, 3, 4

1Agroscope Reckenholz Tänikon, Research Station ART, Zurich, Switzerland


The major findings of our study were that CNT inhibited growth of green algae, but not due to direct effects, like reduced photosynthetic activity. The press discussed the results very diversely: Taking over our message to interpretations far away from the scientific truth, we put forward the idea that an improved understanding of the mechanisms behind the effects of CNT should be promoted. The press release was a good opportunity to reach the public. However, the media is not always a reliable mirror of scientific communication. Therefore, we need to keep in mind that each press release is a new attempt to introduce our message into the public discourse.

RA08-1

The way back from the ivory tower - Environmental sciences should impact public and policy

F. Schwab1, 2, 3, 4

1Agroscope Reckenholz Tänikon, Research Station ART, Zurich, Switzerland

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sediment concentrations of HCB (HCB) and Cadmium (Cd) in sections of the Elbe River basin significantly exceed maximum allowable concentrations established by the European Commission. Even though concentrations of both contaminants have decreased significantly over the past 15 years, levels remain elevated. In addition, previous studies have documented that downstream transport of contaminated sediment occurs primarily during high water events. Given anticipated climate change induced changes in mean discharge, potentially including an increase in the magnitude of high and low water discharge events, this study seeks to evaluate the role of climate change in the long-term fate and the transport of coarser sediments and associated contaminants, a hydrodynamic and cohesive sediment transport model for a 230 km segment of the Middle Elbe River was developed. Of particular interest is contaminant transport to floodplains and retention time in the numerous groyne fields that line the banks of the Elbe River. Multiple climate change scenarios involving changes in the amplitude and phase of yearly streamflow were used to simulate contaminant transport throughout the Elbe River basin at 25 and 50 years in the future. Integration of modeling output with the results of recently conducted (2010, 2011) sediment data enabled an enhanced understanding of current and potential future dynamics of HCB and cadmium transfers in the Elbe River modeling results, challenges simulating transport in groyne fields, techniques for resolving data gaps, and broad-scale suitability of the model to European river basins are discussed.
Risk communication, especially to the general public and end users of plant protection products, is an important challenge. Currently, much of the risk communication the general public receives is via the popular press, and risk managers face the challenge of presenting their decisions and their scientific basis in an understandable way.

Therefore, we decided to explore the obstacles in risk communication, as done by expert risk assessors and managers. Using the discourse analysis framework we have studied perspectives of three stakeholder groups - regulators, industry representatives and academics across Europe.

We conducted 30 confidential interviews, which comprised 15 open-ended questions. A part of the interview guide was focused on communication of pesticide risk to the general public and the ideas experts in the field of risk assessment/management hold of the public perception of pesticides. We employed the key informant approach in recruiting our participants. They were first identified as key stakeholders in ecological risk assessment of pesticides and then sampled by means of a snowball sampling.

The most visible motif we identified in the preliminary analysis was a strong disconnect between “emotions” and “science” in communicating risk. These two were seen as opposites, with “science-based” risk communication perceived as superior to the “emotion-based” communication. The latter was presented as detached from facts and related to political sensitivities.

Our results show that despite the attempt to polarise emotion-based and science-based communication, emotionally charged language and reactions are always present in risk-related topics. Our preliminary findings suggest that there needs to be a balance of “emotions” and “science” in communicating risks - the discourse of risk is tightly related to emotional reactions. Moreover, studies into risk perception found that emotional reactions often play a more significant role in perceiving risks than our cognitive judgement.

 Whilst it is important to keep the process of risk assessment objective and science-based, the general public can be better informed from communicating risks with the costs and benefits clearly and emotionally charged, and emotional reactions have been successfully used in health risk communication (e.g. fear appeal) and public awareness campaigns. It seems that risk assessors and managers can also learn from these examples.

Reach Regulation: communication behind the information needs

Reach Regulation: Communication Behind the Information Needs. The implementation of REACH aims a high level of protection of human health and the environment. Industry has the burden to prove the safety use of products and to ensure control of any risk. If the information to public is also one of the major goals of the regulation, the first steps of implementation show different ways to experience T.

Challenges of integrating science and people within a network of excellence

In parallel the demand for scientifically sound and transparent information upon which to base consumption choices and behaviours is growing among policy makers, REA meetings have been organised with the parties who have designed the regulation and who are assuming the control of its implementation, but also people who are implementing REACH in professional organisations and private companies. Reach regulation designers, chemical producers, chemical industry trade-unions, state-member representative. Information on/into Reach, at this early stage (registration), is not a simple question and Reach proceeds by ‘Learning by doing’. Information on REACH and products is circulating to some extend into topical focused forums (SIEF) and into chemical consortia, but it appears that different stakeholders don’t share the same points of view (what kind of questions for which recipients?). Communication to public which is also an important objective of the regulation is far beyond what maybe expected at that stage.

The next steps (evaluation, authorisation) surely need to plan communication towards the public, outside from the closed and confined space of acting stakeholders.

Research findings and decision making: the case of renewable energy

Scientific research can have a role in the promotion of more sustainable patterns of consumption and production because it can provide information aimed to raise awareness about the impacts of different behaviours and to support more sustainable choices from different kind of actors. The challenge posed to science in this context is to provide information that is effectively supporting for decision making processes at different scales and that can be easily understood by all the stakeholders involved in the process (policy makers, entrepreneurs and citizens). In recent years the attention of citizens on the issues of sustainability, environmental impacts and sustainable behaviour has grown considerably [1]. In parallel the demand for scientifically sound and transparent information upon which to base consumption choices and behaviours is growing among citizens.

Nonetheless, it is difficult to think that there may be a direct contact between those who do research and who makes the decisions: firstly, because in most cases there are no opportunities for direct contact (e.g. to citizens) and, secondly, because is necessary to translate the information resulting from scientific research in a language that is understandable. The lack of communication between science, policy and citizens communication can lead to not evidence-based decision making, lack of trust and unsustainable behaviour due to low level of information and awareness. One possible way of success in environmental communication could be represented by the presence of those intermediares who have relationships with key stakeholders and are able to translate information for them so that they become understandable and translatable into action.

We present a discussion about wood use as a renewable energy source: communication of the main scientific findings about its sustainability and environmental impacts associated to it in support to decision making in energy planning and energy use.

Challenges of integrating science and people within a network of excellence

The German Federal Environment Agency runs a site for aquatic simulation in the very south of Berlin, which also includes a set of outdoor and indoor artificial pond and stream mesocosms (FS) for ecotoxicological research (http://www.umweltbundesamt.de/wasser-und-gewaesserschutz/fsa/). Up to the present, 30 studies were carried out in that facility during the last 10 years. The results have been popularised via the official homepage, guided tours, conference posters, talks and sessions, scientific journals, magazines, newspapers, and TV features. In search for further means to reach a wider audience apart from ecotoxicological professionals and people interested in natural sciences, the idea of the artist Anne Rinn (www.anne-rinn.de) to stage an exhibition and to create a film entitled ‘Keep Your Boots Muddy’ was supported by the FSA team. Both exhibition and film pivot on the triangle nature-simulation-art with simulation trying to create artificial nature. The presentation will be the 10 minutes version of the German film with English subtitles.

RA09 - Focal species of birds and mammals and their ecological behaviour for refined risk assessments of plant protection products in Europe

Benefits of EFSA Risk Assessment for birds and mammals’ guidance document for ecological refinements

Both exhibition and film pivot on the triangle nature-simulation-art with simulation trying to create artificial nature. The presentation will be the 10 minutes version of the German film with English subtitles.
Risk assessment of birds and mammals exposed to plant protection products in the Nordic and Baltic countries

RA09-2
Risk assessment of birds and mammals exposed to plant protection products in the Nordic and Baltic countries

1Swedish Chemicals Agency, Sundbyberg, Sweden
2Rifcon GmbH, Hirschberg, Germany
3State Plant Protection Service, Riga, Latvia
4State Plant Service, Vilnius, Lithuania
5State Plant Service, Sundbyberg, Sweden
6Finnish Safety and Chemicals Agency, Helsinki, Finland
7State Plant Protection Service, Riga, Latvia
8State Plant Service, Vilnius, Lithuania
9Norwegian Food Safety Authority, Ås, Norway

European Birds

Regulation 1107/2009 regarding placing of plant protection products on the market in EU entered into force 14 June 2011. A central aspect of the regulation is the principle of mutual recognition between zones with comparable conditions. To facilitate work-sharing within the Northern zone, the Nordic and Baltic countries have regular meetings to harmonize risk assessments. Agreements among the countries are compiled in a guidance document for the Northern zone which is available at the authorities’ homepages.

To harmonize initial risk assessment of birds and mammals, the Northern zone has agreed on using the EFSA Guidance document (EFSA Journal 2009; 7(12):1438). If initial assessments indicate potential risks, refined risk assessments can be provided. Refinement options include which species that could be used (focal species) and the ecology of these species, e.g., proportion of diet obtained from treated area (PT) and composition of diet obtained from treated area (PD). Other refinement options consider the properties of the substance, e.g., degradation time in/on treated crops and weeds (foliage DT50).

To harmonize a refined zonal risk assessment, the Northern zone has agreed upon the following:

Default foliage DT50 used in initial risk assessment could be replaced if results from at least 4 study sites are available. If results from 4-10 sites are presented, the longest DT50-value should be used. If more than 10 values are available, the mean value can be used. Residue decline studies may only be used for refinement of DT50 if results are evaluated according to the EFSA guidance document. Default PT (100%) could be replaced by referring to studies including, as a minimum, 10 individual animals. The 90th percentile PT value from these studies should be used in the refined assessment. Studies to refine the PT may only be used in the risk assessment if they are evaluated according to the EFSA guidance document.

Focal species and their PD and PT will be harmonized, as far as possible, for major crops within the zone. Such guidance will enhance uniform and agreed refined assessments and ease evaluation of registration reports. Our present data suggests that this guidance and refined risk assessment will be available in May 2012 when the SETAC conference is held.

Modelling (body burden or ecological models) are not considered appropriate for higher tier risk assessment until validated models and guidance are available.

RA09-3
Identification of bird focal species in France for refined risk assessments for plant protection products

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National Museum of Natural History, Paris, France

The refined risk assessment for birds and mammals might be based on different parameters such as measured residues in food items, and/or on behaviour and diet of focal species. The EFSA Opinion (2009) provides generic focal species for the major crops, but when further refinement is necessary, focal species defined in the opinion as “real species that actually occur in the crop when the pesticide is being used” should be identified. The National Museum of Natural History in France has got a huge data base containing registrations of bird presence on the territory, based on field observations made by a large network of naturalists. A methodology has been built to identify the focal species on several groups of crops by crossing the information issued from the Museum database, the repartition of crops in France, protected studies provided by unofficial data from the National Museum.

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RA09-4
Bird focal species in crops according to EFSA - how to find the right candidates

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Syngenta, Bracknell, United Kingdom

National Museum of Natural History, Paris, France

The purpose of the presentation is to demonstrate how an evaluation of the distribution of focal species from these studies with the distribution for the same species provided by the European Birds Atlas database. To illustrate this approach, one crop example (cereals) will be used. The approach developed may allow extrapolation in line with the Zonal Approach, such that MS risk managers can have confidence in using refined exposure data for a single set of focal species applied to a crop within a zone or even across zones.
Mercury emissions in large Hg-polluted floodplain areas in Europe are an understudied problem: challenges for reliable risk assessments and implications for authorities.

Mercury emissions in large Hg-polluted floodplain areas in Europe are an understudied problem: challenges for reliable risk assessments and implications for authorities. Current needs for reliable risk assessments, the induced implications for authorities, and future challenges will be discussed. The presented data will contribute to a better understanding of seasonal dynamics of Hg fluxes and its controlling factors. This presentation should be of large interest for a wide international audience, such as environmental scientists and managers, applied ecologists, and authorities.

Recent studies on distribution and cycling of mercury and a mass balance were implemented. It has been shown that exchange with the atmosphere is the most important source of mercury for the water compartment. Measurements have shown that the evasion of Hg varied between the different seasons with the highest evasion during the autumn and an estimate of yearly evasion from the Mediterranean Sea surface was estimated to range between 250-350 kMol/yr. Two important zones of MeHg productivity, which is typical of the Mediterranean region, were identified.

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It is well known that an antagonistic effect exists between selenium (Se) and mercury (Hg), and that Se can play a protective role against Hg toxicity in organisms. In order to find an evidence for such connection, total Hg and Se were determined in 1654 umbilical cord blood samples, 1081 maternal blood samples, and 1051 breast milk samples in pregnant women from Slovenia, Croatia.

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organisms from all trophic levels from primary producers to fish. Carbon and nitrogen isotopes were determined with a isotope ratio mass spectrometer (IRMS). Total Hg was analyzed using thermal combustion method (AMA 254). The Hg species were analyzed by species-specific isotope dilution and capillary gas chromatography hyphenated to inductively coupled plasma mass spectrometry (ICP-MS). Based on δ²⁰⁴N results the sampled organisms covered 3 trophic levels. Total mercury concentrations in biota varied by nearly three orders of magnitude from 0.030 μg g⁻¹ d.w. (dry weight) in seagrass (Zostera marina) to 2.3 μg g⁻¹ d.w. in tissue of shrimp (Palaeomon elegans). There was a tendency of increasing Hg concentration with increasing trophic level, but the relation was not significant if all species were considered. MMHg concentrations varied between 3 (seagrass, phytoplankton) and about 2000 ng g⁻¹ d.w. (shrimps, fish) and the proportion of MMHg in total Hg was increasing with the trophic position of organisms. For the full set of data, the relation between MMHg and δ²⁰⁴N values was best expressed by an exponential function (R²=0.59). On average, the total amount of Hg and MMHg in the organisms in a moderately polluted area of the Lagoon of Venice increased by one order of magnitude for each of the three trophic levels. However, the deviations from this rule can be considerable for benthic filter feeders (bivalve mollusks) and some fish apparently migrating from less contaminated areas. Results of this and follow up studies should provide a suitable model for risk assessment in more contaminated areas.

RAI-05

Dietary selenium at environmental concentrations reduces methyl mercury retention in some aquatic organisms at the lower trophic levels

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Methyl mercury biomagnifies along aquatic food chains and top predators - including humans with a high intake of fish or aquatic mammals - risk neurological symptoms because of the high dietary exposure to methyl mercury. Methyl mercury is taken up into organisms quite efficiently, whereas - once assimilated - methyl mercury is eliminated slowly. Thereby, the long retention times (of course together with the efficient uptake) are the main contributing factors in the biomagnification process. Selenium interacts with methyl mercury and mercury in multiple, complex and not fully understood ways. Methyl mercury contents in fish tend to be low in selenium rich (natural, manipulated or polluted) ecosystems and dietary selenium increases the elimination of methyl mercury from freshwater fish in laboratory experiments. Lower retention times in organisms at the various trophic levels will inevitably result in reduced biomagnification of methyl mercury along aquatic food chains but the role of dietary selenium on methyl mercury biokinetics in aquatic invertebrates is poorly known. The present experiments were carried out to obtain a better understanding of selenium’s role for the retention of methyl mercury in aquatic invertebrates.

Dietary selenium (as selenite, seleno-cystin and seleno-methionine but not selenate) reduced the retention on methyl mercury in the brown shrimp Crangon crangon. Environmentally relevant concentrations (< 1 μg Se/g) of selenium in the food correlated negatively with half lives for methyl mercury. Selenium in the food also reduced the retention times for methyl mercury in the marine copepod Acartia tonsa, but in a less pronounced way than in the brown shrimp. In shore crabs Carcinus maenas no effect of selenium was found. The finding that there is a negative correlation between low, environmentally realistic selenium concentrations in the food and the half life for methyl mercury in brown shrimps indicates that selenium may play an active role for the biokinetics of methyl mercury in the environment. This is corroborated by similar findings in zebrafish Danio rerio [1] where small increases in the selenium concentration in the food also lead to a dose-dependent decrease in the retention of methyl mercury. The potential significance of the somewhat more limited effect of selenium at the lowest trophic levels (the copepods) and lack of effect in the shore crab needs more detailed elucidation.

RAI-06

Mercury pollution in china: releases, uses and impacts

T. Larsen

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China currently has the world's largest intentional consumption as well as unintentional environmental release of mercury (Hg). Atmospheric emissions has been estimated to about 700 tons annually, accounting for one third of the global anthropogenic emission. There are also large (but not quantified) releases to local soil and water environments. The intentional use of Hg in industrial processes and consumer products has been estimated at 1000 tons annually, roughly half of the global total. Mercury is released to the environment by a wide range of sectors, including those involved in the mining, power generation, non-ferrous metal production, and the cement and chemical industries. The industrial use of mercury in China has caused severe pollution incidents in the past. Today, as a result of past practices, high mercury levels are found in water, soil and rice near abandoned mercury mining and smelting areas. The presentation gives an overview of the major issues regarding China's Hg pollution issues, including releases, intentional use, environmental concentrations as well as human exposure.

RAI-11 - Guidance documents and guidelines for environmental risk assessment (ERA): needs, developments and progress

RAI-11-1

Ecological risk assessment of pesticides: linking non-target Arthropod testing with protection goals (ESCORT 3)


Dow AgroSciences, Monheim, Germany

The presentation gives an overview of the major issues regarding China’s Hg pollution issues, including releases, intentional use, environmental concentrations as well as human exposure.

RAI-11-2

Development of OECD guidance on the conduct and evaluation of toxicity tests for endocrine disrupting chemicals

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The science of endocrine disrupting chemicals (EDCs) is emerging, and two jurisdictions (European Union and United States of America) have now enacted regulations for the control of these substances. It was foreseen over a decade ago that such regulations would need to be underpinned by new toxicity assays relevant to human health and wildlife species, because the more traditional suite of internationally-standardised assays was insufficiently sensitive to known EDCs. In response to this perception, the Organisation for Economic Cooperation and Development (OECD) set up its Endocrine Disrupter Testing and Assessment (EDTA) programme to develop a framework for the control of EDCs and establish a test battery for EDCs, several of which have now been published as OECD Testing Guidelines (TG). These include 2 in vitro procedures for detecting substances with potential to affect steroidogenesis (TG 456) or interact with the estrogen receptor (TG 453); 4 in vivo mammalian assays (TG 443, 441, 407 and 440) with diagnostic capability for, and/or sensitivity to, one or more of estrogens, androgens, thyroid disrupters and steroidogenesis disrupters (so-called EATS modalities); 4 in vivo fish assays (TG 229, 230 and 234; GD 148), also with EATS sensitivity; and one in vivo amphibian assay for thyroid-active substances (TG 231). In addition, OECD has developed, or is developing, a range of lifecycle or partial lifecycle assays with birds, fish,
amphibians and invertebrates that are able to measure the apical effects of EDCs without generally being able to diagnose causality. As well as briefly describing these assays, this paper will present a new OECD Guidance Document (GD) which helps users of the assays interpret their results, assists in reaching conclusions about whether test substances possess potential or actual endocrine disrupting properties, and provides advice about a possible further testing step should this be indicated. The basis of this GD is the need to evaluate all significant information in a weight-of-evidence approach when interpreting assay results, including existing data on similar chemicals, physico-chemical properties, results of in vitro tests, and in vivo toxicity in the same and other species. It will rarely be possible to conclude that a substance is an EDC solely on the basis of a single assay, so the GD will provide much-needed assistance to chemical companies and regulators alike.

RA1A-1
ECETOC Ecotoxicological assessment of endocrine disrupting chemicals
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3Syngenta Product Safety, Jealott's Hill International Research Centre, Bracknell, United Kingdom
4ECETOC, Brussels, Belgium
5Wetano environment, Faringdon, United Kingdom

European regulations on plant protection products, biocides and chemicals only support the marketing and use of chemical products on the basis that they do not induce endocrine disruption in humans or wildlife species. An ECETOC task force was formed to provide scientific criteria that may be used to identify and evaluate endocrine activity. This presentation focuses on wildlife species.

For ecotoxicological assessments the key considerations include specificity, potency, population relevance and negligible exposure. Assessment of specificity is conducted within a study or species, to determine whether the endocrine mediated effect occurs at concentrations lower than those that cause other significant toxicity. Then the evaluation proceeds to consider specificity in relation to endpoints obtained in other taxonomic groups, which may drive the overall risk assessment. If the adverse effects are considered not specific the risk assessment is based on the non-endocrine endpoint. If specificity is confirmed, the endocrine-mediated NOEC/NO(A)EL needs to be compared with other endpoints, e.g. by assessing the magnitude of the ACR, comparing the potency of the substance to a reference compound, duration of exposure that induces an adverse effect and the number of species in which the adverse effect is demonstrated.

The protection goal of environmental risk assessments is the population and examples for relevant effects are: age at first reproduction, size of a reproductive event, frequency of reproductive events, duration of reproductive period, viability of young and sex ratio. Some effects are known to be responsive (and even sensitive) to, but not diagnostic of, endocrine modulation (e.g. fecundity, which can be affected by general toxicity). In such cases supporting information will be required to link the population relevant effect to an endocrine mechanism.

There are currently no specific criteria for "negligible exposure" of wildlife species to plant protection products. Based on the wording in the regulation it is evident that negligible exposure must fall somewhere between "no exposure" (i.e. nominal concentrations of 0), or less than the limit of detection/limit of quantification) and a concentration representing an acceptable or low risk.

RA1A-4
Environmental Quality Criteria (EQC): a comparison of methods under different regulatory regimes
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2Society for Institutional Analysis (sofia), Darmstadt, Darmstadt, Germany

The Federal Environment Agency induced a project comparing the methods under the European REACH-Regulation with national established guidelines for water, soil and the UNECE concept for terrestrial ecosystems. The comparison included the legal frameworks and procedural mechanism for quality assurance. The methodological analysis compares the two frameworks, case studies and interviews with officials to identify gaps in environmental risk assessments and the significance for environmental risk assessments is estimated. In addition, the significance for environmental risk assessments is estimated. In addition, the significance for environmental risk assessments is estimated.
Pyrethroids are synthesized derivatives of pyrethrins, which are natural insecticides that are produced by certain species of chrysanthemum. In the last decades, they have been used as broad-spectrum insecticides because of their low mammalian toxicity, rapid killing action, and ease of use. Pyrethroids exhibit a large variety of possible structures and consist mainly of two isomers. The more frequent isomers are 1R,2S and 1S,2R. Pyrethroids are synthesized from natural pyrethrins, which are also synthesized from pyrethrin I and pyrethrin II. Pyrethrin I is produced from (S,R) or (R,S) stereoisomers, and pyrethrin II is produced from (R,R) or (S,S) stereoisomers. The isomers have different properties, and isomerspecific enrichments in human breast milk were found. This means that they may be constantly present in the body, and even the isomerspecific enrichments in breast milk were observed. Pyrethroids are also used in a variety of products, such as pesticides, insect repellents, and household products. Pyrethroids are also used in the laboratory for their antimicrobial properties.

Pyrethroids have been shown to be persistent in the environment, with some studies reporting half-lives of up to 120 days. This persistence is due to the fact that pyrethroids are not readily degraded in the environment, but rather are transformed into more persistent compounds. These persistent compounds can then accumulate in the environment and be taken up by organisms, leading to bioaccumulation and potential toxicity.

However, until now no harmonized concept for the assessment of the mixture toxicity of biocidal products has been developed. This means that each pyrethroid has its own concept for considering the mixture toxicity during ERA. But this is problematic due to the mutual recognition of authorisations among the Member States. Therefore the German Federal Environment Agency (Umweltbundesamt, UBA) proposes a tiered approach for the assessment of biocidal products based on the available data for the single product components which also considers the existing approaches of the other MS as well as possible synergistic effects between the product components.

The aim of the proposed approach is to harmonize the assessment of the mixture toxicity of complex biocidal products among the MS as well as to assess the mixture composition of products and, where relevant, of ecologically relevant mixtures, and at the same time relieve the data requirements for the applicants as well as additional animal experiments.

RA12

Prioritisation of biocidal substances for environmental monitoring

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The European Biocidal Product Directive (BPD, 98/8/EC) causes a change of the use of biocidal active substances in EU member states. The placing on the market of a number of substances has already stopped or they will be withdrawn soon as consequence of non-inclusion decisions. Additionally, the use of certain biocides will be restricted by mitigation schemes. The expected result of both measures should be a decrease of discharges into the environment of affected biocides. This hypothesis may be proven by an environmental monitoring which might also provide information on risk mitigation measures. Therefore, a project was initiated by the Federal Environment Agency of Germany to develop a concept for the selection of biocides for such a monitoring. First, the status of biocide monitoring in Germany was investigated. A questionnaire was sent to authorities involved in routine monitoring as well as to research groups which run projects in this field. Evaluation of about 25 answers revealed that the current monitoring mainly covers surface waters. Often biocidal compounds are monitored due to legal requirements (Water Framework Directive or German Surface Water Ordinance priority substances). However, most of the biocides currently considered are those which are also used as plant protection products. As result of the survey and an additional literature research a database with monitoring data was compiled. Then, a concept was suggested to prioritise biocidal substances for an environmental monitoring. In a first step compounds are evaluated for emission characteristics (mainly based on intended use in BPD product types). The second step covers potential effects. The scores from both steps are combined and used to prioritize compounds. In a third step it is evaluated in which environmental compartment a compound should be investigated e.g. water, sediment, biota, soil). This evaluation is based on use patterns (product type specific emissions) and substance specific properties relevant for the compartment regarded (e.g. partition between compartments, persistence or BCF). The procedure was tested with a set of 80 biocides which are either already authorised biocides (BPA Annex I) or candidates (biocidal substances currently in the BPA review programme). The plausibility of the prioritisation is discussed with regard to the compiled monitoring data as well as to prioritisation results from other studies.

RA14

Pyrethroid new contaminants in human breast milk

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Pyrethroids new contaminants in human breast milk. Pyrethroids, which are natural insecticides that are produced by certain species of chrysanthemum, are synthesized derivatives of pyrethrins. Pyrethrins are compounds that have been shown to have insecticidal properties. Pyrethroids have been used as pesticides and have been detected in human breast milk.

The study was conducted by the Environmental Research Institute of the University of Stuttgart, Germany. The study was conducted on breast milk samples collected from women who had given birth in the previous 3 months. The samples were analysed for the presence of pyrethroids using gas chromatography and mass spectrometry. The study found that pyrethroids were present in the breast milk of 94% of the women studied. The median concentration of pyrethroids in the breast milk was 0.01 ng/mL.

The study also found that the concentration of pyrethroids in the breast milk was higher in women who were breast feeding their babies for more than 6 months. The study also found that the concentration of pyrethroids in the breast milk was higher in women who were breast feeding their babies for more than 6 months.

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A new modeling approach was designed to account for pesticide residues in various food crops as source for human pesticide intake. Modeled residues are compared with measured concentrations of 12 different pesticide-crop combinations and correspond well with total crop-specific residual errors ranging between a factor of 1.5 for lettuce and a factor of 19 for rice. Intake fractions calculated per unit mass of applied pesticide for 121 substances applied to all crops but potato are usually in the range of 10⁻² and 10⁻³ kg intake per kg applied for typical times between application and harvest. Intake fractions obtained after direct application were 1 to 5 orders of magnitude higher than intake fractions estimated for indirect emissions, i.e. fractions lost to air and freshwater during application. Main factors influencing the fate behavior of pesticides are the degradation half-life in soils and on plant surfaces, the residence time in soil as well as the time between pesticide application and harvest. A simplified model based on the most influential input variables enables the prediction of residues within a factor of 10 of those calculated with the full model. The assessment of the impacts is exposed to the highest assumption of herbicidal crops and fruit trees with usually high intake fractions and consumptions, while roots and tubers only contribute little due to very low intake fractions. Substitution scenarios enable us to reduce health impacts by choosing alternative pesticides with similar ability to control unwanted pests, but with lower toxicity. However, substitution must be discussed separately within each pesticide target class.
and effects of pesticides. In our model the birds are characterised by state variables changing dynamically as an effect of their interaction with other agents, environmental resources and time. The common algorithms (mortality check, managing the energy budget, foraging and conducting seasonal activities) operating on these variables result in reproduction of contaminants to higher organisms has focussed on several environmental factors that may affect food web accumulation. Abiotic factors, species specific traits and habitat have been described to affect accumulation patterns. This implies that spatial variation in environmental factors may play an important role in modulating accumulation. In the current presentation we will present a spatially explicit accumulation model (www.berisp.org), with specific focus on validation of results with field data. The model includes two types of food webs, an omnivorous and an herbivorous one. At the top of the omnivorous food web are two birds species as final receptors: the little owl (Athene noctua) and the blackbird (Turdus merula). Bovine grazer is the consumer in the herbivorous food web. For this study, three field cases were available to validate the models results. For small mammals, data from two case studies are available: the Metalurop Nord area, near a former Pb and Zn smelter in the North of France, and the Afferdensche en Deetsche Waarden (ADW), a metal-contaminated floodplain in the Netherlands. For the bovine grazers, results from a case study from Hageven, Northern Belgium were available. In that study, two herds of cows were followed in a natural area, and their feeding behaviour was monitored. Preliminary results on small mammals show that in the Metalurop Nord case the general pattern of the measured concentrations is reflected in the modelled concentrations: shrub→bank vole→wood mouse→common vole. The differences between measured and modelled concentrations are not significant for the bank vole. For the wood mouse and shrews, however, the modelled concentrations are higher. For the common vole, this is the other way around. Difference between modelled and measured concentrations are not related to systematic model assumptions, but may be explained by differences in the assumed diet. In the ADW case, modelled concentrations in bank voles and wood mice are similar to the measured concentrations, as is also the case for the beetles. In the presentation, more detailed information on other receptors will be provided, including the effect of inclusion of spatially explicit farming.

RA1A-6 Accumulation of trace metals in a complex world, validation of a spatially explicit model: BERISP

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Recently, research on accumulation of contaminants to higher organisms has focussed on several environmental factors that may affect food web accumulation. Abiotic factors, species specific traits and habitat have been described to affect accumulation patterns. This implies that spatial variation in environmental factors may play an important role in modulating accumulation. In the current presentation we will present a spatially explicit accumulation model (www.berisp.org), with specific focus on validation of results with field data. The model includes two types of food webs, an omnivorous and an herbivorous one. At the top of the omnivorous food web are two birds species as final receptors: the little owl (Athene noctua) and the blackbird (Turdus merula). Bovine grazer is the consumer in the herbivorous food web. For this study, three field cases were available to validate the models results. For small mammals, data from two case studies are available: the Metalurop Nord area, near a former Pb and Zn smelter in the North of France, and the Afferdensche en Deetsche Waarden (ADW), a metal-contaminated floodplain in the Netherlands. For the bovine grazers, results from a case study from Hageven, Northern Belgium were available. In that study, two herds of cows were followed in a natural area, and their feeding behaviour was monitored. Preliminary results on small mammals show that in the Metalurop Nord case the general pattern of the measured concentrations is reflected in the modelled concentrations: shrub→bank vole→wood mouse→common vole. The differences between measured and modelled concentrations are not significant for the bank vole. For the wood mouse and shrews, however, the modelled concentrations are higher. For the common vole, this is the other way around. Difference between modelled and measured concentrations are not related to systematic model assumptions, but may be explained by differences in the assumed diet. In the ADW case, modelled concentrations in bank voles and wood mice are similar to the measured concentrations, as is also the case for the beetles. In the presentation, more detailed information on other receptors will be provided, including the effect of inclusion of spatially explicit farming.

RAFT-5 Linking exposure to effects in environmental risk assessment

G. pulex

Chronic effects on pairing behaviour and reproduction of Hyalella azteca following pulse exposure to permethrin

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We performed a 24 hr algae bioassay with synchronized Scenedesmus vacuolatus cell suspensions which were exposed to six concentrations of Norflurazon, Triclosan and N-Phenyl-2-Naphtylamin, respectively. The endpoints cell volume and cell number were measured in a time resolution of two hours within a one-generation algae cell-cycle. A modelled concentration of the toxicant during 10-day exposure for the endpoint survival

G. pulex

To assess the need of TK in survival models, we included or excluded simulated internal concentrations based on pre-calibrated TK. The RA1A-5 - Linking exposure to effects in environmental risk assessment

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Most common ecotoxicological tests assess the acute toxic effects by exposing the organism with high dose and short exposure time under laboratory conditions. Process-based toxicokinetic-toxicodynamic (TK/TD) models may be used for extrapolation to chronic toxic effects under field conditions such as fluctuating and pulse exposures. A TK/TD model simulates the temporal course by linking the toxicant exposure concentration to the observable adverse effect via an internal concentration and effect process in the organism. Various TK/TD models describe the time course of the endpoint survival of an organism. However, only a few TK/TD models exist which simulate the internalisation and/or development of sublethal effects. The objective of this study was the development of a process-based TK/TD model that describes the sublethal effect growth of unicellular algae cell populations exposed to toxicants.

The TK/TD model successfully fitted the averaged algae cell volume exposed to six concentrations of Norflurazon, Triclosan and N-Phenyl-2-Naphtylamin, respectively. This process-based TK/TD model describes the sublethal effect growth of unicellular algae cell populations exposed to toxicants.

We calibrated TKTD models for Gammarus fossarum, exposed to pesticides diazinon, imidacloprid and propiconazole. To calibrate the models, we conducted toxicokinetic experiments (internal and external concentrations measured), acute toxicity experiments (4-d standard LC50 tests) and pulsed toxicity experiments (1-d exposure pulse). 1) We simulated survival of G. pulex in pulsed exposure to propiconazole using a TK/TD model calibrated with acute toxicity data and compared the prediction with experimental results. The comparison showed that the model overestimated mortality in pulsed scenarios, which suggests that acute toxicity data could be used to calibrate TKTD models to achieve protective predictions in pulsed exposure profiles. To assess the need of TK in survival models, we included or excluded simulated internal concentrations based on pre-calibrated TK. The differences between goodness of fits of the models including or excluding TK were small. 2) To investigate whether propiconazole is acting specifically or as a baseline toxicant in G. pulex during toxicity tests internal propiconazole concentrations (ILC50) of known baseline toxicants in Daphnia magna. The internal concentrations of G. pulex reached the ILC50 range of baseline toxicants in D. magna, which indicates that propiconazole acts as a baseline toxicant during 10-day exposure for the endpoint survival. 3) We observed that G. pulex is more sensitive to diathoxin than G. fossarum. Diazinon is an acetylcholinesterase inhibitor and it requires metabolic transformation to diazoxon to inhibit AChE efficiently. When we compare the TKTD parameter estimates of G. pulex and G. fossarum, we see that the elimination of diazonax from G. pulex is much slower, which might explain its higher sensitivity to diazinon.
Pairs of H. azteca were pulse exposed to a single pulse of 1 hour to different nominal concentrations of permethrin; 0, 0.3, 0.9 and 2.7 µg/L. After exposure the pairs were transferred to clean water. During an 8 week test period the treatments were monitored for pairing behaviour and every second week the offspring were counted.

Exposure to pesticides can lead to adverse effects on their survival, the magnitude of which depends on pesticide fate in the environment and the intrinsic sensitivity of exposed organisms. Standard ecotoxological practices calculate the magnitude of these effects based on maintained exposure concentrations using dose-response models. However, in the field exposure patterns are often more complex. In order to take account of such exposure patterns, an approach that includes toxicokinetic (uptake and elimination) and toxicodynamic (internal damage and recovery), TK-TD, can be used to describe processes within exposed organisms. Recovery of populations from adverse effects is used as a proxy for acceptability of some adverse effects on non-target populations. The potential for population recovery depends on toxicant, species specific factors and habitat structure and timing of pesticide applications.

Here we explore the joint impact of intrinsic sensitivity and pesticide specific factors on the population recovery. We developed an individual-based model (IBM) to simulate the joint impact of the subsequent recovery of amphipod populations exposed to four pesticides in different exposure scenarios. Pesticide induced mortality was implemented with two different models. Toxicokinetic-toxicodynamic processes were accounted for by implementing the threshold damage model for survival in half of the treated populations, and those where we used a dose-response model for survival, termed DR-populations. Calculations of the mortality rate in populations exposed to chlorpyrifos, resulted in much more severe mortality in TDM-populations, when compared to DR-populations. These differences resulted in substantial differences in population recovery times. Recovery after 16 day exposure to LC50 of diazinon took the longest, followed by recoveries after chlorpyrifos, carbaryl and pentachlorophenol.

In conclusion, accounting for toxicokinetic-toxicodynamic processes results in differences, based on different pesticide characteristics, in magnitude of mortality and subsequent recovery times and yielded, on average, longer recovery periods.

RA1A-5

Linking pesticide exposure to spatial dynamics: an individual-based model of wood mouse populations

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16RA16-1

Post and pre-authorisation monitoring for pesticides focussing on birds and mammals - recent developments from the SETAC environmental monitoring action group (EMAG)

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Bird and mammal risk assessments for individual pesticides carried out under EU Regulation 1107/2009 routinely use the relevant guidance document issued by EFSA (EFSA, 2009). This guidance outlines procedures of toxicity and exposure assessments for lower tiers. If concern is raised regarding lower tier assessments it may be possible to refine the risk assessment and although a range of options are available, detailed guidance is lacking as regards how to proceed. One possible refinement mentioned in EFSA (2009) is the use of field studies where ‘field studies’ refers both to studies of effects following experimental pesticide applications (i.e. applications made as part of a regulatory study) and also to ‘active monitoring’ of effects following applications of authorised products in agricultural practice. EFSA (2009) also highlights the potential usefulness of ‘passive’ wildlife incident monitoring or surveillance, involving investigation of suspected incidents reported by farmers and members of the public. The Environmental Monitoring Action Group for Pesticides (EMAG-PEST) of SETAC has been investigating the range of both pre and post-authorisation studies that have been conducted as well as the experience gained, with a view to consolidating ‘best practice’.

RA1A-6

Post-authorisation monitoring for mammals, birds and insects - wildlife incident investigation scheme - England

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The Wildlife Incident Investigation Scheme (WIIIS) is a reactive surveillance scheme that monitors the suspected pesticide poisoning of wild animals, companion animals (pets) and beneficial insects. The suspected pesticides include plant protection products and biocides used to control vertebrate pests and similar schemes operate in England, Wales, Scotland and Northern Ireland. WIIIS is partly funded by a levy on pesticide sales in the UK and is co-ordinated by the Chemicals Regulation Directorate of the Health and Safety Executive (HSE), which is the lead authority on pesticide legislation. It has operated within a legal framework since the Control of Pesticides Regulations (COPR) 1986 and given this longevity and funding arrangements, it is probably unique in the world. The results from WIIIS form part of the pesticide regulatory process and are reported quarterly on the internet. The majority of poisonings involve the intentional, illegal use of pesticides and where there is an infringement of legislation on pesticides or the environment, appropriate enforcement action may be taken by the Police, HSE or the local authority. How WIIIS operates and the results of investigations into suspected poisonings in England will be discussed. This will focus on investigations where pesticides are used for an intended purpose. For example, honeybee poisonings from spray applications or feral bee treatments and slug pellet applications and rodent control for incidents with mammals and birds. The extent and possible reasons for the intentional, illegal use of pesticides will also be highlighted. Due to improved analytical methods that were introduced in 2010 (unpublished), WIIIS has reported an increase in the number of bee incidents where multiple pesticides and/or very small pesticide residues have been confirmed. It is unlikely that these were the cause of death of the bees, but pesticides found include thiacloprid, deltamethrin, chlorpyrifos, triclopyr and propiconazole. The agricultural use of pesticides may not be involved in some of these incidents and have wood treatments, amateur garden uses, or pet animal treatments are suspected. An effective, comprehensive and consistent approach to a monitoring scheme is an expensive commitment and a collaborative project among the major disease and contaminant monitoring schemes in the United Kingdom is now facilitated by the Wildlife Disease & Contaminant Monitoring and Surveillance WILDCOMS Network.
As increasing numbers of agrochemicals will not pass this arbitrary assessment step in the future, it is necessary to develop acceptable higher tier methodologies to refine the criteria governing the selection of modelling endpoints, such as the FOCUS Kinetics guidance, have tended to increase the conservative nature of the modelled PECGW.

Semi-field experiments with manual application of insecticide-loaded maize dust in Phacelia (Phacelia tanacetifolia Benth) were set up in semi-field and field trials along the edges of the drilled area (treatment) to study the impacts of dust drift on mortality, foraging activity and brood development in bee colonies (exposure to dust during sowing and contaminated pollen and nectar) using drift-reduced pneumatic sowing techniques. Drilling was conducted during bee flight activity, so foragers were continuously exposed to dust. Two control variants were set up with hives in about 50 m (control) and more than 500 m (remote) distance to the exposed forage plants.

No adverse effect on bees and bee colonies were detected after rape sowing in 2009 and 2011. However, during maize sowing in 2010 and 2011 bee mortality was clearly increased.

The main conclusions indicate that the honey bee can cohabitate with modern agricultural practices provided necessary precautions are taken to maintain viable food resources for bees and avoiding practices that may cause adverse effects. These precautions include the design of agricultural landscapes and the implementation of practices that account for the presence of pollinators. Essential developments also concern the availability of effective and regulated veterinary compounds to help beekeepers eradicate the most important pests from apiaries. An analysis of beekeeping activity in its economical context is also provided. Finally, modern agriculture and beekeeping demands better technical knowledge and a critical lack of training and communication to better accompany the updates in science and technology to the farm and the field is identified.

The case of wild bee species may be considered to be very similar to that of the domesticated honey bee albeit far less well documented. Recommendations are emitted towards all those involved in agriculture, beekeeping regulatory authorities and research, which should be communicated to all as the effectiveness of the actions will rely on their common effort to implement them.
increase challenges due to decreased temperatures. The current study aimed to identify the effects of cold stress on aquatic organisms, which led to increased variability in chemical toxicity.

In the context of environmental conservation, the study of temperature-dependent chemical toxicities in aquatic ecosystems is crucial for understanding the impact of climate change on aquatic life. The research conducted at the Swedish University of Agricultural Sciences and the National Institute for Environmental Studies demonstrated that the sensitivity of aquatic organisms to chemicals differs significantly at different temperature ranges. For example, at optimal temperatures, aquatic organisms exhibited the highest tolerance to chemicals, whereas at lower temperatures, the toxicity increased.

The application of Bayesian networks in the assessment of aquatic risk regions was also highlighted. The use of Bayesian networks allowed for a straightforward calculation of management requirements to reduce risk in each of the six risk regions. By integrating toxicological and ecological concepts, the Bayesian networks provided a more comprehensive approach to assessing risks in aquatic ecosystems.

In conclusion, the study emphasized the importance of considering temperature as a significant stressor in aquatic ecosystems. The findings have implications for the development of water quality guidelines (WQGs) and the management of chemical contaminants. The research underscores the need for further studies to understand the complex interactions between temperature, chemical toxicity, and aquatic life, which is essential for effective environmental conservation and management.
The importance of seasonal variation in the interaction of bioaccumulation and climate change

Workgroup 1 focused on influences on chemical occurrence, fate and bioavailability. Here, I present our results for GCC impacts on bioaccumulation. These impacts occur through three primary mechanisms: influences on environmental exposure, on dietary exposure and on within-organism uptake and loss rates driven by bioenergetic processes. The work focuses on the bioenergetically-mediated impacts of GCC, building on previous modeling of species in the Laurentian Great Lakes.

A bioenergetics model for round goby (Apollonia melanostomus) was parameterized to reflect growth rates observed in Lake Erie, Laurentian Great Lakes. The impacts of climate change on bioenergetics was assessed based on a shift towards warmer annual lake surface temperatures. Simulations were run for the baseline case and for a 1, 2, and 3-degree Celsius increase in annual average temperature. The effects of this warming on chemical bioaccumulation were then estimated by coupling the bioenergetics model to a mechanistic bioenergetic model for a broad range of hypothetical chemicals with log octanol-water partition coefficients (KOW) ranging from 0 to 8 and bioaccumulation half-lives ranging from 0.1 to 1000 days.

The impact of GCC on round goby growth illustrated the non-linear interaction between growth rate and fish thermal range. Two scenarios were chosen to illustrate the impacts of GCC on bioaccumulation: the highest growth scenario, and the warmest scenario. When only annual average concentrations were considered, little difference among scenarios was evident. However, when the seasonal patterns of bioaccumulation were considered, substantial changes, both increasing and decreasing bioaccumulation, emerged. These results, which were not pronounced for metabolizable chemicals than for persistent ones, could be particularly important when they intersect with other time points, such as commercial fishing seasons or critical life stages for toxicological impact. The magnitude of the difference from the baseline case depends most strongly on the biotransformation half-life, and then on KOW.

Additive pressures from herbicides and elevated sea surface temperatures on symbiotic-bearing foraminifera from the Great Barrier Reef

The impact of growing goby reproduction on many marine species. However, such effects have always been in uncontaminated waters. The reality is that many marine species will face the effects of GCC in combination with other stressors.

Chemistry may pose a risk to continued recruitment. This study particularly highlights the importance of addressing the potential for interactive effects between climate change stressors and common marine pollutants.
Heavy oil (HO) pollution is one of the most important environmental issues in the world. In our previous study, we evaluated immunotoxic effects of HO on Japanese flounder (Purpurafugu rubripes) and found that 0.3% of HO led immune suppression at cellular and molecular levels in the fish. Moreover, in the experimental infection with viral hemorrhagic septicaemia virus (VHSV) to the fish exposed to HO, we obtained higher mortalities in dual stressor group (virus infection with HO exposure) than those in control groups.

In this study, we investigated the mechanisms of disease occurrence in the fish given the dual stressor by microarray experiment and dynamic of viral replication in the host fish. After applying stress to the fish by each single stressor or by the dual stressor, the mortalities of the groups were calculated and the liver was dissected for sampling of kidney for the microarray experiment and heart for virus titration. As the results, fifty percent of the fish died in the dual group, while no mortality was observed in single stressor groups as well as in control. These results indicated that the dual stressor induced lethal effect in the fish, even when each single stressor had no effect. To investigate if the mortality in the dual group attributed to viral hemorrhagic septicaemia (VHS), we measured the virus titre in the heart sampled from each group.

The increase of temperature reduced the toxic effect of the herbicide diuron and propanolol at the beginning of the exposure as well as at the final time. In the case of diuron, where NPQ mechanisms were inhibited after 12 hours of exposure, suggesting damage in the pigments where the NPQ takes place.

The interactive mixture toxicity effects of a cyanobacterial stressor and insecticides may partly be grouped according to insect mode of action. Asselman, G. Smagghe, G. Raeymaekers, and B. Smets.

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We investigated mixture toxicity of a harmless cyanobacteria (i.e., Microcystis aeruginosa) and insecticides with different modes of action to Daphnia pulex. The widespread occurrence of such toxic cyanobacterial blooms has become an important concern for aquatic ecosystems. In agricultural areas, these toxins may act in combination with plant protection products, including insecticides. In order to study such combined effects, we exposed the waterflea Daphnia pulex to binary mixture combinations of Microcystis and 8 insecticides for a period of 21 days according to a modified central composite design. Results were statistically analyzed with the concentration addition and independent action reference models to test for additive, synergistic or antagonistic mixture toxicity. Synergistic effects were observed on reproduction for the insecticides carbaryl and M. aeruginosa while fenoxycarb, imidacloprid and spinosad caused synergistic effects on length in combined with M. aeruginosa. Linking the results with the mode of action, we observe no interactive effects in combinations with insecticides affecting the acetylcholine pathway by inhibition (carbaryl and chlorpyrifos). However, synergistic effects on daphnids size are observed with those insecticides that affect the acetylcholine pathway by agonistic or allosteric activity (imidacloprid and spinosad). Although endosulfan has a different molecular target compared with imidacloprid and spinosad, both of these receptors belong to the same superfamily of Cyto-lop ligand gated ion channels. This could explain the common observation of synergistic effects in all combinations with these three insecticides. Combination of tebufenpyrad and M. aeruginosa resulted in antagonistic effects on both length and reproduction. Interestingly, both can affect the oxidative phosphorylation through completely different mechanisms. Tebufenpyrad specifically inhibits electron transport in mitochondrial complex I while Microcystis is known to affect the mitochondria. More research is needed to verify whether the antagonistic effects on reproduction are the results of combined antagonistic effects in the mitochondria. These results suggest a potentially complex interaction pattern between insecticides and harmful cyanobacteria, which may require consideration in future risk assessment of insecticides in the context of climate change.

RA18 - Oil spill effects and risk assessment

RA18-1

Risk of severe oil spills - was deep water horizon an outlier?
P. Eckle, P. Michaus, P. Burgherr, Paul Scherrer Institute, Villigen psi, Switzerland

Following the explosion of the drill rig Deep Water Horizon (DWH) on April 20, 2010 that killed eleven workers, an estimated 670000 tons of oil were spilled in the Gulf of Mexico until the well could be capped on July 15th. This makes DWH the largest single unintentional spill in the history of drilling for oil.

Based on global data of oil spills since 1974, we estimate the expected return frequencies of such very severe oil spill events for different spill sources through the oil chain to answer the question if the DWH spill can be considered an outlier.

This is particularly important in view of the rapid increase in deep and ultra-deep offshore activities, where both a geographical expansion as well as a trend towards drilling at ever greater depths can be seen over the last decade.

We also compare the risk of oil spills from offshore drilling with the risk of spills that is posed throughout the entire oil chain, separately for different infrastructures such as pipelines, tanker ship transport and storage. Data is extracted from our uniquely comprehensive global Energy Related Severe Accident database (ENSAD) that contains accident data from a large number of different sources. The severity is modeled with a generalized Pareto distribution, to measure specifically the risk of very severe accidents.

The results give a mean return frequency based on historical data since 1974 of spills from offshore platforms and rigs exceeding the 670000 tons of the of the Deep Water Horizon oil spill of around 19 years with 5% and 95% quantiles of 7 years and 49 years respectively. Based on this result this accident cannot be considered an outlier.

RA18-2

Which has all the oil gone? Identification and toxicity of oil degradation products
A.G. Scarlett, D. Jones, C.E.W. West, S.J. Rowland

Plymouth University, Plymouth, United Kingdom

Oil is a highly complex mixture containing hundreds thousands of compounds. Recent analyses using two-dimensional gas chromatography - mass spectrometry (GCxGC-MS) of the acid-extractable fraction of oil sands process-affected waters (OSPW) from Alberta Canada has shown that even hydrocarbons thought to be extremely resistant oils and therefore likely also occur in the environment as a result of degradation of oil following accidental spillage. Previously we have reported quantitative structure-activity (QSAR) analysis of both environmental and human endpoints.

Structural class mixtures of individual NA plus a 35-component mixture was tested using the Microtox™ assay. Mussels, Mytilus galloprovincialis, were exposed for 72h to three CNA and their clearance rates measured. The toxicities were modelled for a range of both environmental and human endpoints.

The results of microbial toxicity tests of NA mixtures were consistent with a narcosis mode of action and concentration addition. This suggests that most of the compounds present in OSPW and CNA were contributing equally to their overall narcotic toxicity. The toxicity of CNA to V. fischeri has previously been shown to be greater than that of OSPW. Our tests now show that mussels are more sensitive to CNA than V. fischeri. The models predicted some NA to be several orders of magnitude more toxic to fathead minnows than other structures. In addition these structures were predicted to be endocrine disruptors. When oil degrades in the environment it does not simply disperse. Instead, many hydrocarbons are converted into complex mixtures of acids that are water soluble and toxic to a range of species.

RA18-3

Use of ecosystem service valuation in quantifying ecological impacts and compensatory restoration associated with oil spills in the environment
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Since the Exxon Valdez spill, approaches to quantifying the potential impacts of an oil spill on the environment have evolved significantly. These approaches were developed to the need of the oil producing public to be compensated for the environmental damage. The approaches evolved from a strict monetary compensation framework to an ecosystem service based compensation process. That is, given that there was an impact on the environment, impacts to ecosystem services (ecological and human use services) were to be quantified and then, based upon the lost ecosystem services, the public was to be compensated by the provision of an equivalent amount of ecosystem services through the
The HC50 value (hazardous concentration for 50% of the organisms) and estimated LC50 value for naphthalene, 5.0 × 10^3 µg/l and 5.7 × 10^3 µg/l respectively, were calculated by using a critical body residue for compounds acting by narcosis and a weighted average of log differences between polar and temperate species have little overall effect on sensitivity to oil. As a consequence, toxicity data obtained for temperate organisms may serve to obtain a first indication of the risks in polar regions. Yet, exceptions may result from biotransformation and specific modes of action.

In recent years there has been an increasing emphasis on the significance of invertebrates for assessing environmental impact of oil spills. In the event of accidental oil spills in sub-Arctic and Arctic marine environments planktonic organisms like copepods are realistic targets for oil toxicity based on their significant role in the food web, their abundance and their large lipid content. For many years the SINTEF/NTNU culture of Calanus finmarchicus has functioned as relevant model for standard ecotoxicity testing of North Atlantic crude oils, and a large database of ecotoxicity data exists for this species. Parameterized experimental data on the effects of oil (single oil components, water soluble fraction of oil, and dispersed oil) on copepod survival and reproduction are used as input for development of numerical models for environmental risk and damage assessment. The accessibility of a continuous copepod culture provides homogeneous specimen in terms of developmental stage and lipid content. This, along with sophisticated experimental systems, is a major foundation for investigating effects of stressors on molecular systems. A 15K oligonucleotide microarray has been developed based on 260,000 ESTs sequenced using FLX454 technologies. Several methods, like 1H-nuclear magnetic resonance and mass spectrometry, have also been developed and applied in order to investigate metabolic profiles and alterations. Together these complementary methods give valuable supporting information to well known fitness-related endpoints, as they contribute with modes of toxic action of stressors, and output data may also be used to determine effect limits. The presentation will include results from an experiment aimed at comparing the effects of chemically and mechanically dispersed oil, their modes of toxic action, and proposed effect limits of toxicity based on fitness-related endpoints as well as molecular profiling. These data will provide input to environmental models for risk and damage assessment following acute oil spills.

The sensitivity of polar and temperate marine species to oil components in relation to accumulation kinetics

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Temperate and polar marine species sensitivity to oil components in relation to accumulation kinetics

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Integrating marine physics, biological and eco-toxicological models into a unified simulation system for oil spill risk and impact assessment

M. Reed, D. Slagstad1, F. Vikebø1, J. Juelius1, I. Durgurt1, U. Broman1, R. Nepstad1, O.J. Broch1, M. Alver1, C. Kloek1

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6IMARES, Denhelder, Nederland

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A 3-year project called SYMBOSES has been initiated to develop a holistic, integrated modeling framework for ecosystem-based risk and impact assessments. The strategy is to create existing numerical models, each of which has been previously developed, validated, and proven useful. This approach avoids the cost of basic code development and allows us to address different challenges in that the various components are based on quite different numerical methods. The system consists of physics, biological, oil/chemical fates and ecotoxicological components. The physics models are Eulerian, as are some of the biological models, whereas the pollutant fates and some of the biological components are Lagrangian. The related process equations of the component models are solved in general on different time-steps, and at different spatial resolutions. The design of the system requires the possibility of two-way communication between model components (e.g. zooplankton feeding on phytoplankton, zooplankton grazing on phytoplankton, and dispersal of appropriate species), allowing growth of phytoplankton, mortality, growth and reproduction of zooplankton, and growth and mortality of ichthyoplankton. It is also desirable that alternate components can be inserted into the system, to facilitate geographic transportability as well as uncertainty analyses. We therefore seek a relatively flexible and transparent set of methods to achieve integration of the separate models into the system. We assume that the basic physics models are always Eulerian; regardless of numerical solution, the computations are carried out on a regular or irregular grid that is spatially fixed for the duration of a given simulation. The biological and oil/chemical transport and fates models may be either grid-based or particle-based (pseudo-Lagrangian), and will in general produce data on spatial scales different from that of the physical environment. This paper will describe the proposed design plan to meet these challenges.
are consistent with their inputs (drift-overspray) in the first meter of a field margin directly adjacent to a field under Good Agricultural Practices.

To detect the effects of the applications vegetation assessments and a photo-documentation of the flower intensity of R. acris was performed in May 2010 and 2011. Additionally the experiment was accompanied by monitoring of R. acris in field margins in the study area in May 2011. R. acris revealed subtle effects after the herbicide applications. Flower intensity was significantly reduced in the herbicide treated plots, whereas plant presence was not affected. So far these sublethal effects are not accounted for in risk assessment procedures. However in the long run these effects will cause the decline of specific plants and set in community shifts in agricultural field margins. This was confirmed by the monitoring where R. acris could hardly be observed in field margins adjacent to cereals, whereas in field margins located next to pastures or orchards (no herbicide use) the species was recorded more frequently. Besides the implications for the plants the described sublethal effects can also cause secondary effects for flower visiting arthropods and thereby negatively affect the biodiversity of the agricultural landscape.

RA1-9
Enhancing risk assessment by using a toxicokinetic and toxicodynamic (TK/TD) growth model of Myriophyllum spicatum
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Toxicological studies used for risk assessment can only cover a limited range of exposure scenarios due to their work intensity. Ecological models considering the toxicokinetic and toxicodynamic of chemicals are capable to close the lack of exposure scenarios in toxicological studies and, thus, enhance risk assessments by predicting the effects of variable exposure scenarios.

In this work a toxicokinetic and toxicodynamic growth model of Myriophyllum spicatum is presented giving the ability to predict the effects of chemical substances on the growth of M. spicatum. Therefore, a growth model considering the most important factors influencing the growth was developed. A toxicokinetic part calculating the uptake and distribution of chemicals depending on their physicochemical properties and the physiology of M. spicatum was included in the growth model. Moreover, a toxicodynamic part using the internal concentration in M. spicatum and experimentally established dose-response relationships to modulate the growth rate of M. spicatum was added to the model. The model is able to predict reversible growth inhibition under different environmental and exposure conditions based on standard test results.

The model was verified with experimental data on growth inhibition of M. spicatum after fourteen days due to different concentrations of 3,5-dichlorophenol (DCP). The model was able to predict the effect of 3,5-DCP exposure on M. spicatum very well but cannot fully explain the total inter experiment variability of growth, particularly under untreated conditions. The model can be used to predict growth inhibition for 3,5-DCP. Further validation and verification of the model is needed to ensure that the model works properly and to show that the model can be used for a wide range of substances with different physico-chemical properties.

RA1-9.3
Herbicide impacts on macrophytes: can we predict community-wide effects from single-species toxicity tests?
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1University of Toulouse, Toulouse, France
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RA1-9.4
Linking aquatic plant toxicity data for pesticides to risk assessment endpoints and environmental protection goals
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Compliance Services International, Rochester, Ma, United States of America

This presentation discusses protection goals for aquatic plants, assessment endpoints that address those protection goals, and the relationship of standard toxicity data to the assessment endpoints and protection goals. Protection goals for aquatic plants generally focus on the plant community as a whole, rather than particular species or individuals as is the case for aquatic animals. Consistent with these protection goals, it is not necessary to completely protect every sensitive plant species; protecting some fraction of plant species (represented by a certain percentile of the Species Sensitivity Distribution, SSD) is sufficient to protect overall community function and structure. Assessment endpoints used to characterize effects of chemicals on aquatic plants should represent the impacts of similar severities to assessment endpoints used for animals, in relation to their respective protection goals. Toxic endpoints for aquatic plants are based on non-lethal responses, unlike acute endpoints for animals which are based on mortality. Furthermore, aquatic plant communities usually recover more quickly than animal populations (especially fish) from effects of stressors. These considerations suggest that assessment endpoints for aquatic plants should use higher SSD percentiles than the 5% typically used for animals. When data are unavailable for a sufficient number of species for SSD analysis, a small number of surrogate test species (e.g. R. acris, Myriophyllum spicatum, and certain algae) can indicate the toxicity of herbicides and fungicides to the most sensitive aquatic plants.

RA1-9.5
Uptake and intra-cellular accumulation of hydrophobic chemicals in charophytes and implications for ecosystem exposure control and remediation
S.C. Schneider, L. Nizzetto
Norwegian Institute of Water Research, Oslo, Norway

Uptake to primary producers (constituting an important pool of organic carbon in lakes) is a key step for addressing hydrophobic chemicals to upper trophic levels. It has been hypothesized that permeation and diffusion through biota surface structures such as epilitha, cell walls or membranes may be inhibited for highly hydrophobic substances by steric hindrance of membrane passage. Chara rudis is a macroscopic, perennial, benthic taxon having a protective layer of cortex cells. We used 14C12 radiolabelled Hexachlorobenzene (HCB) to i) investigate whether or not HCB can reach the internode cell, ii) to assess the distribution of HCB in different plant parts, and iii) to discuss the implications of the accumulation on benthic macroalgae for fresh water ecosystems. We found that i) about 65% of the total amount of HCB added to the microcosm was associated to the algae tissues, ii) the HCB in the cortex cells represented about 90 to 95% of the total found in the plant, and iii) HCB was detected at measurable levels in 57% of the sampled internode cell cytoplasm, demonstrating the occurrence of intracellular transport and storage of chemicals. In some water bodies charophytes grow in very dense meadows up to 2m tall and can cover a large fraction of the water body bottom. The high efficiency of taking up hydrophobic chemicals directly from the water phase suggests that they also can represent a key control for the mass balance of POPs in the water ecosystem wherever they are abundant.

RA1-9.6
Halophyte filters: the potential of using halophyte species for phytoremediation purposes in saline aquaculture
H.J de Lange, M.P.C.F Paulissen, P.A. Slim
Alterra, Wageningen UR, Wageningen, Netherlands

There is a growing consumer demand for seafood as protein source, and land-based (marine) aquaculture is seen as a sustainable way to meet this growing demand. A major problem with aquaculture is the nutrient waste, since most of the nutrients added through feed are released into the environment, predominantly in dissolved form. Filtration and sedimentation techniques to reduce nutrient concentrations are not adequate for the large volumes of waste water produced in aquaculture. The use of constructed wetlands has been shown to be successful in freshwater aquaculture practices. Freshwater constructed wetlands are worldwide used to treat various types of wastes. However, there is only limited experience in saline systems. The main aim of this study is to analyse the potential of constructed saline wetlands in land-based marine aquaculture. The main advantage of using wetlands is that these are relative simple systems that need little control after construction. The location of the wetland can be located close to the source, limiting transport of waste water through pipelines. Further, wetlands can simultaneously reduce several contaminants (BOD, suspended solids, nutrients, pesticides, pathogens). The primary function of a constructed wetland is the purification of water, but there are also several ancillary benefits that can be incorporated in wetland treatment designs. For example a high vegetation biodiversity, offering a habitat for fauna, and giving aesthetic, recreational, commercial, and educational human uses. Different approaches exist to utilize plants (halophytes, macro-algae, micro-algae) in the treatment of marine aquaculture effluent; these are described in a conceptual framework. In essence, the approaches attempt to maximize the ecosystem service of water purification. The approaches differ in the balance between economic benefits and nature benefits. The potential use of this framework is demonstrated with a Dutch case study.

RA2-0
Risk assessment of chemical mixtures: where do we stand? what are the next steps?

RA2-1
Predictive regulatory risk assessment of chemical mixtures in the aquatic environment: a conceptual framework
M. Faust, T. Backhaus
1Faust and Backhaus Environmental Consulting GbR, Bremen, Germany
2University of Gothenburg, Göteborg, Sweden

 качественные и количественные надежности используются для оценки качество-составления. Например, в случае катализаторов, часто используется оценка сезонные “кокетки”. Мы использовали два метода для оценки мальтиной точки концентрации (CA) и независимого действия (IA) для развития иерархического подхода к оценке среды и риска оценки смесей, фокусируя на общеобразовательные и химические регулирования в ЕС под REACH и предполагая, что такой “базовый” набор данных доступен, например, EC50 значения для аллогенов, гидратов, и рыб. Как мишени точнее оценивается у предсказания CA, таких как примущие неравильно первичный иерархические отказ от риска действия агентов в смеси.
components. In particular, we show that summing up PEC/PNEC ratios might serve as a justifiable CA-approximation, to extrapolate from the "base set" data to the aquatic ecosystem in a regulatory first tier assessment. This approach makes optimum use of already existing single substance assessments. More in-depth mixture investigations may be requested only if the first tier estimates give an indication of a potential environmental risk. Finally we suggest to call for mode-of-action driven analyses only if error estimations indicate the possibility for substantial differences between CA- and IA-based assessments.

RA2-02

Bearing down the borders? - Considerations on an environmental risk assessment of substance combinations across different regulations
Federal Environment Agency, Dessau-Roßlau, Germany
Across Europe, the prospective environmental risk assessment (ERA) of chemical substances is conducted under separate regulatory frameworks, i.e. for plant protection products, biocides, industrial chemicals as well as human and veterinary pharmaceuticals.
The aim of this prospective assessment is to avoid adverse effects of these chemicals prior to an exposure of the environment. Under all regulations, the current assessment methods focus on single substances only. As it is well accepted that multiple substances typically reach environmental compartments together and act jointly on organisms, strategies and methods for an assessment and regulation of chemical mixtures are currently under development for all these regulatory frameworks.

The presentation focuses on the activities at the "Chemical and Biological Safety Division" of the German Federal Environment Agency (Umweltbundesamt, UBA) with a focus on the development of common methods and strategies for the consideration of substance combinations under the different legislations. Furthermore the limitations and needs for an assessment of mixtures that reach the environment from multiple sources, i.e. substances that traditionally fall under different legislations, are pointed out. Existing approaches for overarching concepts beyond substance-oriented regulations are discussed from an regulatory perception. Common agreements on definitions, assessment methods and strategies across the substance-oriented regulations are important. The same applies for an exchange of knowledge and data between regulations, which would enable an assessment of substances from a variety of sources. Overarching concepts might be helpful for certain situations and substances, but it needs to be clarified for which cases and who might conduct such an assessment.

RA2-03

The threshold of (eco)toxicological concern: a suitable tool for mixture risk assessment and ranking?
T. Backhaus
University of Gothenburg, Gothenburg, Sweden
The threshold of toxicological concern is defined as a safe level: a concentration at or below which there is no appreciable risk for humans (TTC) or the environment (ecotTC). Recently, the TTC concept has also been put forward as a tool for the risk assessment and ranking of chemical mixtures, for example in the recent opinion on mixture toxicity assessment by the scientific committees of the European Union. Particularly it has been argued that compounds that are present at or below their individual (eco)TTC do not contribute to the (eco)toxicity of a mixture. That is, a mixture would pose no risk for human health or the environment, as long as all components are present at or below their individual (eco)TTC.

The TTC approach has a range of appealing features. It would be extremely helpful for simplifying the (eco)toxicological assessment of complex mixtures by allowing to ignore compounds below their individual (eco)TTC. It would also simplify the problem of mixture toxicity assessment to a single substance assessment, as we can safeguard against any mixture effect, as long as we can ensure that the concentrations of all compounds never exceed their individual (eco)TTC. Furthermore, the (eco)TTC seems to provide a risk basis for the exposure-based waiver of risk assessment in the context of e.g. REACH. A first ecoTTC-like approach is already implemented in the form of the so-called action limit in the European guideline for the environmental risk assessment of pharmaceuticals.

I will briefly review the conceptual basis of the (eco)TTC, the suggested applications in the context of mixtures toxicity assessment and will then discuss the implications of its use change over time, (ii) the (eco)TTC concept needs to be adjusted for mixtures that contain at least partly compounds with a similar mode of action, which might make it primarily useful for risk management, and (iii) that in particular the ecoTTC requires further development and refinement if it is to be used in the context of ecological protection goals.

RA2-04

Long-term trends in potential toxicity of unknown organic micro-pollutants in rivers
J. Struys, E.V.D. van der Grinten, T. Aldenberg
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During these years, the effects of toxic substances on the ecosystem of Dutch inland waters were measured with a complementary method, i.e., by means of so-called bioassays. This approach provides information on the effects of unknown chemicals in water which are overlooked by traditional analytical techniques. The latter cover only a small portion of the large number of chemicals that are present in surface water. Moreover, classical chemical techniques do not give insight into the auxiliary effect that several toxic substances may have.

The results from the bioassays confirm that damage to the aquatic ecosystem during the last decade due to the presence of toxic substances has decreased. Toxic pressure in the water of the river Meuse and Scheldt was significantly higher than that in the river Rhine, but has decreased considerably since the year 2000. The results also indicate that the toxic pressure is higher upstream and decreases downstream.

The reaction of five organisms to toxic chemicals in a water sample was measured with bioassays. Applying the species sensitivity distribution method to this small set of data results in a snapshot of the toxic stress. This approach is however flawed by large uncertainty margins which make a series of snapshots unsuitable for discerning a trend in water quality. A trend became apparent when all bioassay results collected over the whole period were combined by means of an advanced statistical technique. As a result, information on toxic metal mixtures is provided that is based on exposure-based waiving of risk assessment in the context of e.g. REACH. A first ecoTTC-like approach is already implemented in the form of the so-called action limit in the European guideline for the environmental risk assessment of pharmaceuticals.

RA2-05

Toxicity of metal mixtures to Daphnia magna: Implications for a multi-metal, multi-site biotic ligand model
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In real-world aquatic systems, organisms are usually exposed to metal mixtures instead of individual metals. To predict the toxicity of metal mixtures, we have been developing a mechanistic model based on tissue residues of metals and on the concept that the toxicity of a mixture of metals can be either dose-additive or response-additive, depending on the mechanisms of action. To calculate tissue residues and thereby predict toxicity across wide ranges of water quality, we have been developing a multi-metal, multi-site biotic ligand model (MMSB LBM) that concurrently accounts for metal-metal competition for binding on dissolved ligands in the water and at sites of toxicity on organisms. We have exposed Daphnia magna to mixtures of Cu and Zn, Cd and Cu, or Cd and Zn in moderately hard reconstituted water containing dissolved organic matter (added as Suwannee River fulvic acid) at 3 mg DOC/L, and compared observed mortality to the null assumption of additive mortality predicted from results of concurrent Cd-only, Cu-only, and/or Zn-only toxicity tests. This research has revealed several apparent metal-metal interactions that otherwise might lead to conclusions that metals interact in non-additive ways, yet simple geochemical speciation in the BLM can explain these interactions and reconcile the apparent non-additive toxicity. For example, the toxicity of Cu-Zn mixtures always appeared to be more-than-additive or additive when based on dissolved metal concentrations, whereas Cu was variably more toxic when based on tissue concentrations.

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RA2-06

Low dose mixture effects - detectable using toxicogenomic approaches?
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The advent of toxicogenomic techniques have raised large expectations that some of the central questions of mixture toxicology such as to use novel perspectives if not definite answers. After the first decade of experimental studies this review summarizes mixture toxicology studies that
address diagnostic, mechanistic or extrapolation questions. Since 2002 almost 40 studies were published with their major focus on mixture toxicity assessment by means of toxicogenomic techniques, mainly through microarray or qPCR techniques, though metabolic and proteomic analysis of joint exposures have also been undertaken. It is now standard to explicitly state criteria for selected concentrations and provide insight into employed data transformation, and statistical treatment with respect to minimising sources of bias and to biomolecular analysis of toxicogenomic data, by contrast, is still a field with diverse and rapidly evolving tools. The combined effect assessments achieved are discussed in the light of established toxicological dose-response and mixture toxicity models. Often transcriptomic responses are discussed based on the presence or absence of signals. As there are no consent ways of how to interpret these effects, there are ambiguous interpretations. Furthermore, mixture studies in their majority designed their experiments and compared their recorded outcomes against individual treatments i.e. focus was to receive signals of individual components under mixture exposure. This may in turn contribute to our understanding of existing interconnections and apparent apical combined effects. Here models are employed to calculate expected combined effects based on information of the mixture components' individual dose-response relationships. By joining these mixture effect models with toxicokinetic and -dynamic thinking we suggest a theoretical framework that may help to overcome the current limitation of providing mainly anecdotal evidence on mixture effects and progress into more hypothesis driven mixture studies. As ways forward we suggest to study and establish quantitative relationships between dose dependency and time dependency of responses.

RA21-1 - Standard vs non-standard methods for hazard and risk assessment

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The use of outbred laboratory animal strains representing wild populations is often advocated but rarely demonstrated in ecotoxicology. Due to practical constraints limiting effective population sizes, laboratory animal strains are generally more inbred and less genetically diverse than their wild counterparts. Furthermore, breeding history may have a profound impact on inter-population variability and ultimate phenotypes used for assessing effects of chemical exposure. Developmental and toxicological phenotypes are particularly susceptible to variation due to phenotypic plasticity caused by genotype[GERKX]environment interactions. Sexual selection in the zebrafish is a potential case in point. Sex determination in this species has been shown to be highly plastic and therefore influenced greatly by environmental factors (including chemical exposure), as well as genetic factors. We compared a range of phenotypic endpoints in zebrafish from four different “breeding treatments” comprising a WIK zebrafish strain and a WIK/Wild strain with three levels of inbreeding (FIT=n, n+0.25, n+0.375) in the new OECD234: Fish Sexual Development Test (FSDT), where one of the core endpoints is sex ratio. There were no differences between treatments in terms of egg viability, hatch success or fry survival. However, compared with WIKs, WIK/Wild hybrids were significantly larger in size with more advanced gonadal (germ cell) development at the end of the test. Increasing the levels of inbreeding in the related WIK/Wild lines did not affect body size, but led to a male-bias in sex ratio of the wild-type line (FIT=n+0.375). Conversely, in the reference WIK strain there was a significant female-bias in the population (80% females). Overall, our results support the use of outbred zebrafish strains in the FSAT. Despite increased variance in some endpoints, WIK/Wild outbreds (FIT=n) met all acceptance criteria for controls, whereas WIKs failed to comply with tolerance limits for sex ratio (30-70% females). Sexual development was also more advanced in WIK/Wild outbreds (6-7 days). WIK was allowed to be used for dose-dependence studies with wild-type and outbred zebrafish strains, as well as for testing OECD test guideline 402. Several other OECD guidelines are affected by the use of outbred zebrafish strains in ecotoxicology is generally lacking. This additional information should aid further standardisation of regulatory tests.

RA21-2 - Improved protocols for sediment toxicity testing: a review

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Recently, several standardised protocols for the in situ toxicity of sediment-bound chemicals to sediment-dwelling organisms are relatively well established for a few test species. However, there is a lack of cost-effective and widely accepted methods to assess potential effects on microorganisms, macrophytes and animals across taxonomic groups, as well as methods to translate results of such tests between freshwater and marine ecosystems and to the population and community levels. Such methods, however, are crucial within the current context of criteria setting and prospective risk assessment (RA). Here, we critically review the state of the art on prospective sediment toxicity testing with individual organisms and microorganisms to identify known gaps and to provide guidance for future sediment toxicity test designs for microorganisms, macrophytes and benthic invertebrates. Focus is on freshwater, estuarine and marine systems in the temperate zone, however also with relevance for other climate zones. We synthesise an overview of recommendations from the literature on sediment preparation and spiking, microorganism, macrophyte and invertebrate tests, microcosms and mesocosms in the regulatory context and framework of PRA. Standard test protocols are only available for benthic invertebrates, whereas a protocol for the freshwater macrophyte Phragmites australis sp. is currently under development. Tests for macrophytes, invertebrates and microorganisms to a lesser extent are described in the literature but prospective micro- and mesocosms tests are rare and highly diverse. Compared to freshwater sediment tests, marine and estuarine tests have received much less attention. In order to perform a proper sediment toxicity test, it is recommended to use spiked artificial sediment and artificial water, assess exposure by passive sampling and use selected test species. Standard species together with optimised standard test protocols form the basis of an improved first tier of sediment PRA.

RA21-3 - Use of public and dossier data in WFD EQS derivation compared to risk limit derivation in other regulatory frameworks

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In the Netherlands, RIVM has a broad experience on both the derivation of Environmental Quality Standards (EQSs) under the Water Framework Directive (WFD) and the evaluation of dossier data within various regulatory frameworks, not only because of different ways of literature searching, but also because of diverging choices made in assessing toxicokinetics and -dynamic thinking we suggest a theoretical framework that may help to overcome the current limitation of providing mainly anecdotal evidence on mixture effects and progress into more hypothesis driven mixture studies. As ways forward we suggest to study and establish quantitative relationships between dose dependency and time dependency of responses.

RA21-4 - Standard and non-standard ecotoxicity tests in regulatory risk assessments of chemicals

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Guidance documents recommend the use of standard ecotoxicity tests in regulatory risk assessments [1-6]. These recommendations have decreased the use of non-standard test methods for several groups of chemicals. On the positive side, this has increased the reliability of the data since the strict reporting requirement for standard tests enhances reliability. On the negative side, it could have implications for the relevance of the risk assessment. Standard tests may not always be sensitive enough to measure the specific effects expected for instance from endocrine disrupting chemicals, nanoparticles and pharmaceuticals. Bisphenol A is an illustrative example since three standard tests are preferred by several risk assessors over 200 non-standard studies with lower effect values [7]. Previous studies indicate that ecotoxicity studies published in the open scientific literature are surprisingly often insufficiently reported [8]. This may be a major reason why they are often seen as less reliable in a risk assessment context. Choice of reliability evaluation method could also affect the outcome of the evaluation [8]. Several guidance
documented recommendations that the method described by Klimisch et al. [9] should be used for evaluating the reliability of ecotoxicity and toxicity data but the Klimisch criteria give a strong preference for standard tests.

We present a novel and more comprehensive method for evaluating and reporting non-standard ecotoxicity data [10]. Its aim is to enable an increased use of non-standard data in risk assessments. As part of this work we clarify the definitions of "reliability" and "relevance" in order to promote the consistent use and application of these concepts in risk assessment procedures. Lastly we present examples from environmental risk assessments of pharmaceuticals showing how non-standard data can complement standard data to arrive at robust and transparent risk assessments.

RA21-5
Toxicity and detoxification of chemicals in detergents, softeners and shampoos
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Previously environmental concern was mainly focused on the intentional release of chemicals from use of pesticides and discharges from industries. Presently concern has also been raised on non-intentional release of chemicals from articles and products like paints, textiles, tires, plastics, pharmaceuticals, and personal care and cleaning products. For all chemicals their hazard and risk is based on toxicity (including genotoxicity and sensitation), degradation and biodegradation, either from real testing or chemometric modelling. However, for products and articles, which may contain many chemicals at confidential concentrations, assessments of biodegradation, biocumulation and combined toxicity based on ingredient toxicity are not possible to make. Therefore, an approach similar to that used for industrial wastewaters using toxicity testing and, when necessary, TIE (Toxicity Identification Evaluation) might be used. Since many chemicals will reach the environment via sewage treatment works, their biodegradation and detoxification are important for their environmental ecotoxicity. Research on detergents, softeners and shampoos has shown that surfactants are key chemicals for the toxicity of these products, and that there is a wide range in product toxicity, as well as in their detoxification. Recent studies, using the standard ISO test with Daphnia (ISO 6341) and innoculation by activated sludge, have shown that toxicity (24 h EC50) have ranged from 4 to 1615 mg L-1 for 26 detergents, from 25 to 225 mg L-1 for 5 softeners, and from 0.54 - 163 mg L-1 for 9 shampoos. This variation suggest that there is a considerable potential for toxicity reduction among these products either through replacement of entire products or by chemical substitution. The former needs more transparency of environmental labelling and a more sophisticated labelling into more than one category. The latter implies a better communication and co-operation between academia, industry and regulators. Recent research on detoxification has shown a wide range also in detoxification and differences between products in abiotic (without activated sludge) and biotic (with activated sludge) degradation. Even if the reasons for all variation is not known it is obvious that this aspect should be considered together with toxicity in the risk and hazard assessment.

RA21-6
Aquatic toxicity of halogen-free flame retardants
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Research on evaluating the environmental impact of halogen-free flame retardants (HFFRs) has only recently become topical, and a lot of data are currently being gathered. The alternative flame retardants studied included six organic phosphates (Aluminium Dihydrophtal Phosphate (ALP), Bispheonol A bis(diphenyl phosphate) (BDP), Dihydro Oxa Phosphaphenanthrene (DOP0), Melamine Polyphosphate (MMP), Resorcinol bis(diphenyl phosphate) (RDP), Triphenyl Phosphate (TPP) and six inorganic compounds (Ammonium Trihydroxide (ATH), Ammonium Polyphosphate (APP), Antimony Trioxide (ATO), Magnesium Hydroxide (Mg(OH)2) and Silicon Dioxide (ZS)). A modelling approach was used to study the transference of the ARs into the environment during AR treatments for Norway rat control. Baiting with brodifacoum resulted in lower levels of AR entering the food chain via the rats and lower numbers of live rats carrying residues of AR during and after the trials due to its effectiveness against resistant rats. Bromadiolone and difenacoum resulted in markedly higher levels of AR uptake into the rat population in Germany and leucine120glutamine in England. A modelling approach was used to study the transference of the ARs into the environment during AR treatments for Norway rat control. Baiting with brodifacoum resulted in lower levels of AR entering the food chain via the rats and lower numbers of live rats carrying residues of AR during and after the trials due to its effectiveness against resistant rats. Bromadiolone and difenacoum resulted in markedly higher levels of AR uptake into the rat population and more live rats carrying residues during the trials and for long periods after the baiting period. Neither of these compounds provided full control on any of the trial farms. Secondary non-target predators were predicted to take up more AR when residues in rats were higher and the more rats remained alive. In resistant areas where ineffective compounds are used there is the potential for higher levels of AR exposure to non-target animals, particularly predators of rats and scavengers of rat carcasses. Thus, resistance influences the total amount of AR available to non-targets and should be considered when dealing with rat infestations, as resistance-breakers may present a lower risk to wildlife.

RA22-2
What do anticoagulant rodenticide residues in predatory birds and mammals tell us about non-target exposure and risk?
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Anticoagulant rodenticides (ARs) are non-specific in their toxicity and so wildlife species that have been measured in non-target species to gain information on scale of exposure and to assess the likelihood of effects. The second-generation anticoagulant rodenticides (SGARs) have been a focus for monitoring because their widespread use, high acute toxicity and persistence enhance potential for secondary exposure and poisoning. We review what monitoring has told us about the key factors that mediate exposure of non-target species and the likelihood of adverse effects.
Exposure of predatory birds and mammals to SGARs is widespread but species vary in their accumulation of liver residues. Diet is assumed [but rarely shown] to be a major determinant of differences in residue accumulation occur between species with similar diets. However, a recent study strongly suggests that diet can indeed be a key factor. When prey guild is restricted, foxes (Vulpes vulpes) feed relatively extensively on commensal rodents and other species likely to take AR bait. As a result, they accumulate greater SGAR residues than animals from areas where prey choice is wider and includes species unlikely to encounter bait. Usage is also likely to influence exposure. At large spatial scales, the overall pattern of wildlife exposure reflects SGAR usage but the relationship between use and exposure appears more complex at a local scale (414). In addition, other than the total amount used, is likely to be of importance. Resistance to ARs in commensal species may also affect exposure in predators. Resistant rats survive (and are available to predators) for longer than non-resistant individuals and may also accumulate higher AR body burdens. The importance of resistance in mediating exposure and risk remains a key question. It can be addressed through analysis of wildlife residues but first requires that resistance areas are mapped. The relationship between magnitude of liver residue and likelihood of mortality in wildlife is poorly defined. A "potentially lethal range" has been proposed for barn owls (Tyto alba) but is poorly defined, not diagnostic and may vary between ARs and species. New probabilistic analyses to assess the likelihood of mortality associated with any given AR residue offers an exciting new approach that may allow, for the first time, estimation of likely toxicity at a population level and identify inherently sensitive species.
RA23-3
Reflections on the risk of first generation Anticoagulant Rodenticides to Raptors
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In 2008, the United States Environmental Protection Agency placed new regulatory restrictions on the use of second generation anticoagulant rodenticides. These changes are expected to result in expanded use of older first generation indandione compounds (FGARs; e.g., diphacinone, chlorophacinone). Recent acute oral and 7-day dietary diphascione toxicity studies conducted in American kestrels (Falco sparverius) and Eastern screech-owls (Megascops asio) suggest that raptors are considerably more sensitive to FGARs than predicted by data developed with traditional avian wildlife test species (Northern bobwhite, Colinus virginianus and mallards, Anas platyrhynchos). Many studies have suggested that the toxicity of FGARs to target rodent pest species is more pronounced in a multi-day exposure scenario. Likewise, some data indicate that the toxicity of FGARs to non-target wildlife is greatly enhanced in a repetitive dietary exposure scenario. Regulatory agencies continue to require and use acute oral toxicity data (i.e., LD50 derived from single or multiple doses administered in a 24-hour period) as a significant component of their ecological risk assessments. While a valuable measure of toxicity, the LD50 can underestimate the toxicity of FGARs that can be more toxic when consumed over several days. Additional information examining the effect of repeated frequency and duration of FGAR exposure would improve risk assessments. Furthermore, sublethal FGAR responses (e.g., bleeding, coagulopathy, histopathological lesions) that may constitute biologically significant adverse effects are considered, but are given less weight in the overall risk assessment. Some of these adverse effects could affect survival of free-ranging birds in ways that were not apparent in a controlled laboratory setting. Choice of test species, laboratory exposure regimens and toxicity endpoints, as well as better characterization of the probability of exposure in field situations, deserve further attention to more accurately assess the risk that FGARs pose to non-target wildlife.

RA24-2
Investigating the potential risk of secondary rodenticide poisoning to urban owls inhabiting and foraging in urban landscapes of the Lower Mainland, British Columbia, Canada
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Anticoagulant rodenticides are widely used to control pest rodents, but poisoning of non-target wildlife has been linked to these practices, including secondary poisoning of birds of prey, particularly owls. In this study, we investigate whether Barred owls (Strix varia), Great-horned owls (Bubo virginianus) and/or Barn owls (Tyto alba) inhabiting and foraging in predominantly urban landscapes of the Lower Mainland, British Columbia are at risk of consuming rodenticide-laden prey, such as rats and house mice. By conducting a pellet study, we found that urban Barred owls had the largest proportion of rats in their diet, with some individuals’ diet consisting primarily of rats. Urban Great-horned owl pellets were also comprised mainly of rats, but there was a clear shift towards alternative prey base when urbanization within home ranges decreased. Field voles (Microtus cotocotus) were the main prey item for Barn owls, regardless of the amount of urbanization within their home range. For all three species, consumption of rats and house mice appears to coincide with increased urbanization within home ranges. The shift in the diet of owls living in urbanized areas may potentially lead to an increased risk of secondary rodenticide poisoning. Radio telemetry was deployed to further investigate which landscape features urban Barred owls select as foraging habitat. This information will further our understanding of the interactions between urbanization and the foraging activity of owls. To date, the study has demonstrated that the toxicity of FGARs to non-target wildlife is greatly enhanced in a repetitive dietary exposure scenario. Regulatory agencies continue to require and use acute oral toxicity data (i.e., LD50 derived from single or multiple doses administered in a 24-hour period) as a significant component of their ecological risk assessments. While a valuable measure of toxicity, the LD50 can underestimate the toxicity of FGARs that can be more toxic when consumed over several days. Additional information examining the effect of repeated frequency and duration of FGAR exposure would improve risk assessments. Furthermore, sublethal FGAR responses (e.g., bleeding, coagulopathy, histopathological lesions) that may constitute biologically significant adverse effects are considered, but are given less weight in the overall risk assessment. Some of these adverse effects could affect survival of free-ranging birds in ways that were not apparent in a controlled laboratory setting. Choice of test species, laboratory exposure regimens and toxicity endpoints, as well as better characterization of the probability of exposure in field situations, deserve further attention to more accurately assess the risk that FGARs pose to non-target wildlife.

RA25-2
How the uses of anticoagulant rodenticides influence the distribution of their residues in rodent community?
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In Europe, the uses of anticoagulant rodenticides (AVKs) are regulated as biocides (Directive 98/8/EC or as plant protection product (Directive 91/414/EEF referred as pesticide uses hereafter). According to these uses, the active ingredients (a.i.) found in the commercial products and the quantities that are applied in the environment may be very different. Biocide uses of AVKs correspond to a large diversity of a.i. (8 molecules are homologated) which are applied around villages and habitations at relatively small quantities locally. Plant protection is authorized with 3 a.i. only and is commonly realized in eastern France with large amount of bromadiolone poisoned baits (up to 20 kg / ha) over vast areas (e.g., 12,000 ha) in 2006 to control Water voles outbreaks. If large mortality events of rodent predators are generally associated to pesticide uses, secondary exposure of wildlife to biocide AVKs is now widely reported. Here, we aim to document the distribution of 8 AVKs in the rodent community according to the uses in eastern France. For each context of use, 2 areas were selected and the localization and intensity of AVKs treatments were characterized as precisely as possible. Then, both target and non-target rodents were trapped in autumn up to 1 km from the place where AVKs treatments have been identified. The residues of the 8 AVKs authorized in Europe were measured in the liver and the whole body of 100 specimens selected in each area. Anticoagulant rodenticides were largely distributed in the rodent community of the treated areas whatever the type of uses. In the pesticide area, a high proportion of individuals in both target and non-target species were exposed to bromadiolone (45%) and the high levels of residues in some individuals (>50,000 ppb in the liver for some Water voles and mice) may explain lethal poisoning of rodent predators. In biocide areas, median liver concentrations of all AVKs were 3.5 and 1.3 ppb in target and non-target species respectively, 23% of the specimens trapped exhibiting detectable residues of AVKs in the liver. This suggests that predators are frequently exposed to low doses of different AVKs but the impact of sublethal exposure of AVKs to rodents remains under question.

RA26-2
Risk mitigation measures for anticoagulants used as rodenticides in Sweden
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Anticoagulants used as rodenticides are non-selective, highly toxic substances. Second generation anticoagulants (SGARs) (bromadiolone, difenacoum, brodifacoum, flocoumafen and diphacinone) are also persistent with extremely slow elimination from the body, and prone to accumulate in non-target species that consume poisoned rodents (secondary poisoning). They have characteristics which meet the criteria to be classified as potential PBT and potential vPvB substances. Furthermore, monitoring studies in several European countries have shown high levels of second generation anticoagulants in predatory mammals, raptors and owls. Nevertheless, SGARs have been included in Annex I to Directive 98/8/EC because of their identified benefits for public health and the lack of established alternatives which are at the same time equally effective and less damaging to the environment. If SGARs are to be used, extensive risk mitigation measures need to be applied in order to reduce the risks for primary and secondary exposure for humans, non-target animals and the environment. The Swedish Chemicals Agency finds it appropriate to restrict authorisations of rodenticides containing SGARs to user category “class 1 - professional use with a specific permit”. In combination with other risk mitigation measures, this is considered an appropriate way to reduce the risks yet allowing for effective rodent control.

RA23-4
Wastewater effluent discharges: chemical characterisation and understanding potential risks in receiving waters

RA23A-1
Wastewater effluent discharges: chemical characterisation and understanding potential risks in receiving waters

The UKWIR Chemicals Investigation Programme
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Recent EU legislation in the field of water and the environment, in particular the Water Framework Directive (WFD), has important implications for the scope and nature of pollution control measures required to protect surface waters. Environmental Quality Standards (EQSs) have been set for substances that hitherto have not been subject to detailed monitoring or control. These standards have, in turn, generated a need for compliance assessment and, where necessary, the development of appropriate control measures. The UK Water Industry has responded to this challenge by collaborating with national environmental regulators to undertake a £25M programme of investigations of contaminants in wastewater, their fate and behaviour in wastewater treatment and their sources within urban sewer catchments. Effort is focussed on a range of over sixty contaminants, including priority substances regulated at European level, specific pollutants regulated at national level and a range of substances, including pharmaceuticals, of emerging importance. The project is intended to identify and prioritise substance categories that are likely to require future action under the Water Framework Directive. It also seeks to establish the sources of different substances and to determine the most effective forms of control, including measures not related to end of pipe treatment. This paper provides an overview of the chemicals investigation programme, describes its key outputs and provides examples of how the results of the programme have been used in order to prioritise proposed future measures to be implemented as part of the WFD. The relative performance of different wastewater treatment processes will be discussed in relation to the achievement of good chemical status for surface waters.
RA23A-4

Assessment of WWTPs efficiency to limit surface water pollution by illicit drugs
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Water contamination resulting from consumption of illicit drugs is a new concern for water management that must be considered, not only because of the social and public health implications but also in an environmental context, because of the contamination of surface waters by partially treated effluents. The discharge of contaminated effluents from wastewater treatment plants (WWTPs) is a concern for water management and the environment.

RA23A-2

PhACs in wastewater treatment and in receiving waters - emerging issues
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Pharmaceutically active compounds (PACs) are emerging contaminants found in surface waters at ppt levels. Thousands of PACs are approved for human/ veterinary use, although only a very small percentage of these compounds have been studied in the environment. Some of the most commonly used PACs are sold in hundreds of tonnes/year in the UK alone. PACs enter the environment mainly through insufficiently treated sewage, effluents from manufacturing processes, runoff and sludge (if used as fertiliser or transported to landfills). They are ubiquitous and persistent (due to their continuous introduction into the environment) with synthetic properties. PACs have also been detected in drinking water, which poses a direct risk to humans and raises the issue of contaminated water sources.

RA23A-1

Occurrence of Pharmaceuticals on a sewage impacted section of a Mediterranean River (Llobregat, NE Spain) and their behaviour under different hydrological conditions
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Mediterranean rivers are characterized by high flow variability, strongly influenced by seasonal rainfall. In addition, the river receives effluent discharges of more than 55 million m³/year, especially at drought periods, the effluents may represent almost 100% of the total flow of the river. When water scarcity periods occur, water flow and dilution capacity of the river is reduced. On the other hand, floods contribute to remobilization of pollutants from sediments. Consequently, the potential environmental risk of pollutants is expected to increase. Besides, due to the high economic activity and intensive urbanization in the Mediterranean region, in Europe, river pollution is frequently higher than in other European river basins.

RA23A-3

Assessment of WWTPs efficiency to limit surface water pollution by illicit drugs
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RA23A-5

PACs' occurrence in wastewater and surface waters - emerging issues
B. Kasprzyk-Hordern

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Pharmaceutically active compounds (PACs) are emerging contaminants found in surface waters at ppt levels. Thousands of PACs are approved for human/veterinary use, although only a very small percentage of these compounds have been studied in the environment. Some of the most commonly used PACs are sold in hundreds of tonnes/year in the UK alone. PACs enter the environment mainly through insufficiently treated sewage, effluents from manufacturing processes, runoff and sludge (if used as fertiliser or transported to landfills). They are ubiquitous and persistent (due to their continuous introduction into the environment) with synthetic properties. PACs have also been detected in drinking water, which poses a direct risk to humans and raises the issue of contaminated water sources.

RA23A-6

Are the concentrations of micropollutants responsible for the reduction in wastewater toxicity for gammarids following the application of ozone?
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Micropolllutants, which comprise among others pharmaceuticals and personal care products, enter the aquatic environment via sewage treatment and wastewater and are thus frequently detected in surface waters. To counteract these micropolllutants, advanced treatment methods are currently under discussion. In this context the application of ozone is considered as an effective tool. However, its ecotoxicological implications are largely unknown. Therefore, the aquatic toxicity of secondary (non-ozone) or ozone treated wastewater was assessed using the feeding rate of the leaf-shredding invertebrate Gammarus fossarum, which is known as a key-species in the ecosystem function of leaf litter breakdown. Two repetitive experiments resulted in significantly higher feeding rates for gammarids exposed to ozone compared to non-ozone treated wastewater sampled from a treatment plant equipped with a full-scale ozonation. While ozone was applied at the lab-scale, a further experiment confirmed the detoxification of wastewater from the same treatment plant following its treatment with ozone. Moreover, the deviations in the dissolved organic carbon profiles of ozone and non-ozone treated wastewater samples were assessed using the feeding rate of the leaf-shredding invertebrate Gammarus fossarum, which is known as a key-species in the ecosystem function of leaf litter breakdown. Two repetitive experiments resulted in significantly higher feeding rates for gammarids exposed to ozone compared to non-ozone treated wastewater sampled from a treatment plant equipped with a full-scale ozonation. While ozone was applied at the lab-scale, a further experiment confirmed the detoxification of wastewater from the same treatment plant following its treatment with ozone.

RA23A-7

Characterising the presence of organic micropolllutants in wastewater effluents is fraught with challenges. The full assessment of the effectiveness of treatment options is often difficult as many organic micropolllutants transform during the treatment process. Thus, a pure target screening approach may indicate that a compound is no longer present following treatment, while the compound is still present but in a transformed state. We show here how this can be addressed using a combination of target and non-target screening approaches. The program enviMass, developed in-house, was used to perform target and non-target screening of wastewater effluents around Switzerland. Between 97 and 111 of 376 target compounds were found and quantified using enviMass, with concentrations from low ng/L (lowest detection limit 5 ng/L) to μg/L. The number of transformation products detected as targets (over 10 per sample of a total of 30 TPs) indicates the significance of accounting for transformation processes in wastewater effluents and thus considering also "unknown" peaks when assessing samples. The number of non-target peaks varied greatly between the treated effluents, as did the presence of isotope peaks, providing some idea of peak identity for positive and negative ionisations. Over 40% of negative non-target peaks had sulfur present, attributed mainly to sulfonic acids. In contrast, many homologous series were detected in positive ionisation samples, generally resulting from peptides. A comparison of peak intensities for target and non-target peaks revealed that of the top 20 peak intensities in negative mode, only 4 were target compounds. These were three artificial sweeteners (acesulfam, saccharin and cyclamate) and a pharmaceutical (diclofenac). The remaining non-target peaks are clearly a significant part of the sample, despite the comprehensive target list of environmentally relevant compounds selected for relevance to Swiss conditions and based on experience. Despite not knowing the identity of the non-target peaks, we show here that we can follow target compounds, transformation products and even non-target masses in wastewater effluents using an inhouse software which is available for public use. The information extracted in the enviMass workflow can be used to quantify target and TPs as well as prioritise non-target compounds for identification and track these peaks in future monitoring programs.

RA23A-9

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RA23A-10

Characterising the presence of organic micropolllutants in wastewater effluents is fraught with challenges. The full assessment of the effectiveness of treatment options is often difficult as many organic micropolllutants transform during the treatment process. Thus, a pure target screening approach may indicate that a compound is no longer present following treatment, while the compound is still present but in a transformed state. We show here how this can be addressed using a combination of target and non-target screening approaches. The program enviMass, developed in-house, was used to perform target and non-target screening of wastewater effluents around Switzerland. Between 97 and 111 of 376 target compounds were found and quantified using enviMass, with concentrations from low ng/L (lowest detection limit 5 ng/L) to μg/L. The number of transformation products detected as targets (over 10 per sample of a total of 30 TPs) indicates the significance of accounting for transformation processes in wastewater effluents and thus considering also "unknown" peaks when assessing samples. The number of non-target peaks varied greatly between the treated effluents, as did the presence of isotope peaks, providing some idea of peak identity for positive and negative ionisations. Over 40% of negative non-target peaks had sulfur present, attributed mainly to sulfonic acids. In contrast, many homologous series were detected in positive ionisation samples, generally resulting from peptides. A comparison of peak intensities for target and non-target peaks revealed that of the top 20 peak intensities in negative mode, only 4 were target compounds. These were three artificial sweeteners (acesulfam, saccharin and cyclamate) and a pharmaceutical (diclofenac). The remaining non-target peaks are clearly a significant part of the sample, despite the comprehensive target list of environmentally relevant compounds selected for relevance to Swiss conditions and based on experience. Despite not knowing the identity of the non-target peaks, we show here that we can follow target compounds, transformation products and even non-target masses in wastewater effluents using an inhouse software which is available for public use. The information extracted in the enviMass workflow can be used to quantify target and TPs as well as prioritise non-target compounds for identification and track these peaks in future monitoring programs.
Could be allowed to be disposed of to river. The most toxic samples were analysed and Phenol and formaldehyde were identified as key contaminants. Their contribution to 2008.

1University of Waterloo, Waterloo, Canada

The response of wild fish to Municipal Wastewater Effluent Exposures at sites in Canada

RA23B-3

The fate of hazardous compounds was assessed in a scale nitrifying sand filter as part of the chemical investigations program, a UK national scheme instigated by the Environment Agency. Risk assessment was conducted with the development of adequate measures to meet the requirements of the Water Framework Directive. Natural and synthetic estrogens are endocrine disrupting chemicals that can cause adverse effects on the sexual and reproductive systems in wildlife, fish, and humans [1]. The nitrifying sand filter examined was a tertiary treatment process designed to remove wastewater solids and perform nitrification by specialized bacteria growing in a biofilm. The sand was continuously fluidized to control the rate of biomass growth and the accumulated solids removed by an airlift system. From the 49 substances investigated a detailed overview on the fate of selected oestrogens: ethinylestradiol, 17β-estradiol and 17α-ethinylestradiol is provided. The wastewater quality, the sand filter performance and operation were also taken into consideration as well as the chemical-physical characteristics of the target compounds.

Several studies have reported a possible connection between nitrification and oestrogens removal [3], others have underlined the relevance of solids retention time [4] and the removal through sorption onto wastewater sludge [5]. The implications of using tertiary treatment processes for hazardous pollutants removal on energy and investment are also taken into account.

Final effluent concentrations were found to be in agreement with the values reported on literature [3]. Removals of E1, E2 and EE2 from the main stream were 89%, 78% and 21%, respectively. For proposed environmental quality standards E2 was always below the reference value of 1 μg/L, average concentration of E1 was below the reference value of 1 μg/L, but EE2 was was always above 0.1 μg/L.

Nitrifying sand filters remain a good candidate for a line process for removal of oestrogens from wastewater although improvements are needed to meet environmental quality standards for the Water Framework Directive.

Reducing and monitoring toxicity in an industrial effluent, using a regulatory approach

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Between 1997 and 2000 the UK government started to try to regulate complex effluents using Direct Toxicity Assessment (DTA). Sites were selected, effluents screened and then ranked for further investigation. This was followed by characterisation, risk assessment, toxicity reduction efforts and monitoring.

In this case study we explore a data set spanning 8 years where we applied these processes for an industrial chemical company, resulting in a collaboration that significantly reduced the final effluent acute toxicity.

Effluents were tested using Tisbe battaglia and Skeletonema costatum bioassays. High toxicity was identified and it was decided that the effluent needed moderating before it could be allowed to be disposed of to river. The toxic samples were analysed and Phenol and formaldehyde were identified as key contaminants. Their contribution to the overall toxicity was calculated using a toxic units approach. Assays were performed according to the UK EAs Standard Committee of Analysts methods and were carried out every month from 2003 until the present. The most toxic samples taken through the industrial process were analysed for phenol and formaldehyde and found to have high concentrations in the 1000s mg/L range. A new piece of equipment was introduced to scrub these specific chemicals from the effluent before it was discharged to the environment.

Following the introduction of the new plant in 2006, detectable toxicity in the two bioassays dropped significantly and has remained low, apart from one unexplained peak in 2008.

This shows the success of the DTA approach and the potential it has to lead to solutions that work for industry and the regulators in reducing toxic inputs to the environment.

Evaluating advanced treatment of hospital wastewater

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This study aims to evaluate the importance of hospitals as point sources of pharmaceuticals into the environment and to propose possible measures to minimize the pharmaceutical input. For that purpose, a pilot-scale wastewater treatment membrane bioreactor (MBR) was operated at a Swiss cantonal hospital over one year. Part of the
MBR permeate was further treated either by powdered activated carbon (PAC), ozone, or photocatalysis (UV/TiO₂). Employing robust sampling strategy and quantifying over fifty highly consumed pharmaceuticals by online SPE-LC-MS/MS provides robust and reliable results. Measurements of species-specific resistance determinants as well as multi-resistant Pseudomonas aeruginosa have shown that hospitals release high amounts of antibiotic resistant and multi-resistant bacteria to the environment. Hospitals are also major contributors to the load of contrast media, some antiinfectives and anesthetics in the environment. Decentralized hospital wastewater treatment is technically possible and ozonation as well as PAC treatment can be recommended as techniques to reduce pharmaceutical load as well as ecotoxicological effects. For most pharmaceuticals high elimination rates were achieved with both treatments; lowest eliminations were observed for the contrast media diatrizoate and ioxitalamic acid. Elimination efficiencies observed in hospital wastewater treatment were similar to the ones in municipal wastewater treatment. Results will be used to support the legal decision whether or not decentralized hospital wastewater treatment would be an option for Switzerland.

RA231B-6
An examination of the toxic properties of water extracts in the vicinity of an oil sand extraction site
Environment Canada, Montreal, Canada
The industrial extraction of oil sands (OS) in northern Alberta, Canada, has raised concern about the water quality of the nearby Athabasca River. The purpose of this work was to examine the toxic properties of various water extracts on Oncorhynchus mykiss trout hepatocytes. The water samples were fractionated on a reverse-phase C18 cartridge and the levels of light-, medium- and heavy-weight polycyclic aromatic hydrocarbons (PAHs) were determined by fluorescence spectroscopy. Primary cultures of trout hepatocytes were exposed for 48 h at 15°C to increasing concentrations of the C18 fraction corresponding to 0.02, 0.1, 0.5 and 2.5X concentrations from upstream/downstream sites in the Athabasca River, groundwater samples, OS tailings and interceptor well-water samples. Changes in cell membrane permeability, activity of phase I and phase II biotransformation enzymes (cytochrome P4501A and glutathione S-transferase activities), oxidative damage (lipid peroxidation LPO) and genotoxicity (single and double DNA strand breaks) were monitored in post-exposure cells. The water samples produced minor changes in membrane permeability but did increase all the above endpoints at thresholds of between 0.02 and 0.1X the water concentration. The most responsive biomarker was DNA damage but it also offered the least discrimination among sites. LPO was stronger at sites downstream of the industrial operations compared to upstream sites. A decision tree analysis was performed to formulate a set of rules by which to identify the distinctive properties of each type of water sample. The analysis revealed that OS tailings and interceptor waters were characterized by an increased concentration in light PAHs (> 42 µg/L) and this fraction represented more than 85% of the total PAHs. These samples also inhibited GST activity, which could compromise the elimination of genotoxic PAHs present in the system. An analysis of groundwater samples revealed a contamination pattern similar to that for OS tailings. There is a need for more research into specific biomarkers of toxicity from OS tailings compounds such as naphthenic acids, light PAHs among others, which are a characteristic fingerprint of OS extraction activities.
MO 001

Calibration and field deployment of five integrative samplers for the monitoring of indicator and dioxin-like PCB (ECLIPSE project)

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Various integrative samplers, at different stages of development, are now available to estimate time-weighted average (TWA) concentrations of freely dissolved hydrophobic contaminants in aquatic environments. The ECLIPSE project (2009-2011, coord. C Miege, Istres) involves 5 laboratories and aims to calibrate and compare 5 different integrative samplers for the monitoring of contamination and dioxin-like PCB in water: semi-permeable membrane devices (SPMDs studied by Istres), low-density polyethylene strip (LDPE, Iffram), silicone rubber (SRPs, Deltares), ChemSorb® (BRGM) and continuous-flow adsorptive sampler (CFIS, LABAQUA).

The first step of the project was the calibration of these samplers under laboratory conditions. During summer 2009, samplers were exposed under constant agitation and temperature in a stainless steel tank filled with 2001 of PCB contaminated water. A constant PCB concentration of about 1 ng/L was achieved by immersing a large water volume, with attached samplers, in the treatment plant (Courbevoie, France) spiked with indicator and dioxin-like PCB. Prior to deployment, samplers were spiked with Performance Reference Compounds (PRC) and exposure durations ranged from 1 day to 3 months. After exposure, processing and analysis, samplers were compared in terms of sampling rate, linear uptake phase duration, repeatability and accuracy of calculated TWA concentration.

The second step of the ECLIPSE project was the deployment and comparison of these samplers in real aquatic environments. In summer and autumn 2010, three field campaigns were carried out along the Rhône River and near the lake Le Bourget (France), known to be contaminated by PCB. Using appropriate cages, canisters and holders, studied samplers were exposed simultaneously at an approximate depth of 1 m during 1, 2, 3 and 4 weeks with duplicates and/or triplicates. These field campaigns allowed to compare in-situ ease of use and robustness of the five integrative samplers in addition to repeatability of sampling and TWA concentration estimate.

MO 002

An international collaborative study on the use of passive samplers in monitoring of emerging pollutants

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The first step of the project was the simultaneous calibration of these samplers under laboratory conditions. The time-integrated data from these devices can be used to complement chemical monitoring of priority and emerging contaminants which are difficult to analyse by conventional conventional spot or bottle sampling methods, and to improve risk assessment of chemical pollution. In order to increase the acceptance of passive sampling technology amongst end users and to gain further information about the robustness of the calibration and analytical steps, several inter-laboratory field studies have been performed in Europe. Such trials are essential to further validate this sampling principle and to improve the confidence of the technological approach for end users. An inter-laboratory exercise on the passive samplers for the monitoring of emerging pollutants was organised in 2011 by the European framework association (Network of reference laboratories for monitoring emerging environmental pollutants; www.norman-network.net) together with the European DG Joint Research Centre to support the Common Implementation Strategy of the WFD. Thirty academic, commercial and regulatory laboratories participated in the passive sampler comparison exercise and each was allowed to select their own sampler design. All the different devices were exposed at a single sampling site to treated waste water from a large municipal treatment plant. In addition, for each target analyte class the organisers deployed in parallel, multiple samplers of a single type which were subsequently distributed to the participants for analysis. This allowed an evaluation of all the components of the different analytical laboratory procedures to the data variability. The results obtained allow an evaluation of the potential of different passive sampling methods for monitoring selected emerging organic pollutants (pharmaceuticals, polar pesticides, steroid hormones, fluorinated surfactants, triclosan, bisphenol A and brominated flame retardants). The exercise was a great learning experience for organizers and participants. The results will be used to inform EU Member States about the potential application of passive sampling methods for monitoring these compounds within the framework of the WFD.

MO 003

Method for passive sampling of TBT in river waters using silicone rubber - field application

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The water replenishment meant that we could be confident that any removal of the contaminant by samplers was not sufficient to significantly reduce the concentration of that contaminant in the water phase (meaning that the concentration could be assumed to be constant). Samplers were removed from the tank weekly and the study was terminated after four weeks exposure. Samplers were then extracted and the extracts analysed for the spiked compounds. Relative uptake rates of various compounds to different samplers were established.

MO 004

Comparative uptake rates of pharmaceuticals and organophosphate flame retardants in a range of passive samplers

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Passive samplers have been widely used to compare contaminants in integratable. In this range of passive samplers (Chemcatcher (polar), Chemcatcher (non-polar), POCIS, SPMD and PDMS (silicone rubber)) were assessed in a laboratory study. The samplers were deployed in a tank with a continuous flow of water from a header tank, which was set at a rate to allow complete replacement of the water in 30 minutes. Into the header tank, a known concentration of chemical was dosed. Compounds investigated were pharmaceuticals and Organophosphate flame retardants.

The purpose of this tank test is to determine suitability of each of the four samplers in the analysis of these compounds, and to establish sampling rates for the compounds of interest.

The water replenishment meant that we could be confident that any removal of the contaminant by samplers was not sufficient to significantly reduce the concentration of that contaminant in the water phase (meaning that the concentration could be assumed to be constant). Samplers were removed from the tank weekly and the study was terminated after four weeks exposure. Samplers were then extracted and the extracts analysed for the spiked compounds. Relative uptake rates of various compounds to different samplers were established.

MO 005

Simultaneous quantitative analysis of pesticides including Phenox-Acids in surface water using UPLC-MS/MS with direct injection

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LC/MS/MS is widely used, in combination with solid phase extraction techniques, to achieve the very low detection levels (low ppt range) needed for the monitoring of pesticides and other pollutants like pharmaceutical drugs in surface and drinking water. Various approaches are possible, depending on the MS instrument, on the needed sensitivity and on the nature of the sample (drinking or surface water). SPE is usually done off-line, starting from a large volume of water (up to 500 ml to 1 l), with associated drawbacks (long protocols, transportation of the samples, cost). Preconcentration can also be done automatically on-line, with different draw backs (long protocols, transportation of the samples, cost). The evolution of HPLC to Ultra Performance LC (UPLC) and new generation of tandem-quadrupole mass spectrometer bring additional possibilities. The analysis of most pesticides is now possible at low ppt by direct injection.

The LC-MS/MS method that we present has been developed for the quantitative analysis of pesticides including phenox-acids. The chromatographic separation is done using a Waters Xevo TQ-S tandem mass spectrometer. The MS detector is a Waters Xevo TQ-S tandem mass spectrometer, with fast polarity switching. For each analyte, the most intense MRM transition was quantified and reported. This method allows the analysis of more than 4 samples per hour. Surface water samples were filtrated, spiked with the internal standard (deuterated compounds) before injection (250µl).

The analysis of more than 25 basic pesticides residues and of phenox-acids was done simultaneously in less than 15 minutes, injection to injection. This method revealed an excellent linearity across the studied range (5-500 ppt) and an excellent repeatability with RSDs typically lower than 2% over 10 injections at mid concentration. Limits of detection range from 5 ppt for the less sensitive compound (MCPB), to below 0,02 ppt for the most sensitive compound (Carbendazim). Despite the very minimum sample
MO 007

Monitoring trials for detection of ionic herbicide residues in water using passive samplers


MO 008

Challenges of passive sampling for selection of sorption materials

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The results show that apolar interaction of charged compounds with the sorption material is an important factor regarding sorption affinity. The more pronounced the apolar moiety is, the higher the sorption coefficients. Equally important is the influence of various inorganic ions in the water phase. This study shows that for e.g. metformin changing from Ca2+ to Na+ the sorption coefficients on a cation exchanger rise by one order of magnitude.

These results help evaluate if the deployment of passive samplers for a compound is useful under certain conditions. It becomes clear that sampling in e.g. Ca2+ rich water is more challenging than in Na+ containing water and that small organic ions with a lack of apolar moieties are difficult to concentrate on a passive sampling material so far.

MO 009

Development of novel monitoring, analytical chemistry and modelling methods for improving the ecological risk assessment of radioactive caesium in the aquatic environment in East Japan

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Polar Organic Chemical Integrative Samplers (POCIS) have been originally developed for sampling polar compounds from the aquatic phase. POCIS consist of an adsorbent material which is an important factor regarding sorption affinity. The more pronounced the apolar moiety is, the higher the sorption coefficients. Equally important is the influence of various inorganic ions in the water phase. This study shows that for e.g. metformin changing from Ca2+ to Na+ the sorption coefficients on a cation exchanger rise by one order of magnitude.

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MO 010

Seasonal monitoring of organic UV filters and UV light stabilizers in coral reef water and beach water by passive samplers

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These charged micro pollutants happen to exist in the environment in large numbers due to anthropogenic activities. Many of these compounds have a high potential to be adsorbed on the surface of seawater and suspended particles. Therefore, sorption materials allowing coulomb interaction are often used to enable sampling of these compounds. Detailed knowledge on their sorption behaviour, however, is sparse, especially concerning passive sampling in environmental monitoring. The exact influence of both the charged and the neutral moiety on the sorption behaviour is not yet well-investigated. The influence of various mineral and organic inorganic ions (e.g. Ca2+, Cl-) that are commonly present in water, on the sorption affinity is just as little understood. The results show that apolar interaction of charged compounds with the sorption material is an important factor regarding sorption affinity. The more pronounced the apolar moiety is, the higher the sorption coefficients. Equally important is the influence of various inorganic ions in the water phase. This study shows that for e.g. metformin changing from Ca2+ to Na+ the sorption coefficients on a cation exchanger rise by one order of magnitude.

These results help evaluate if the deployment of passive samplers for a compound is useful under certain conditions. It becomes clear that sampling in e.g. Ca2+ rich water is more challenging than in Na+ containing water and that small organic ions with a lack of apolar moieties are difficult to concentrate on a passive sampling material so far.

MO 011

Calibration data for Polar organic chemical integrative samplers (POCIS)

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Polar Organic Chemical Integrative Samplers (POCIS) have been originally developed for sampling polar compounds from the aquatic phase. POCIS consist of an adsorbent material which is an important factor regarding sorption affinity. The more pronounced the apolar moiety is, the higher the sorption coefficients. Equally important is the influence of various inorganic ions in the water phase. This study shows that for e.g. metformin changing from Ca2+ to Na+ the sorption coefficients on a cation exchanger rise by one order of magnitude.

These results help evaluate if the deployment of passive samplers for a compound is useful under certain conditions. It becomes clear that sampling in e.g. Ca2+ rich water is more challenging than in Na+ containing water and that small organic ions with a lack of apolar moieties are difficult to concentrate on a passive sampling material so far.

MO 012

In-situ validation of 3 PRCs and 14 pharmaceuticals and 20 endocrine disruptors on the polar C18 Chemcatcher

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In response to the growth of organic pollutants identified in surface water many multi-residue analytical methods have been developed to quantify multi-families of emerging pollutants in water. Many strategies include enrichment steps for water samplings due to very low levels of pollution [1]. Recently, sampling systems more realistic of
environmental exposure have been designed to get time weighted average (TWA) concentrations of those xenobiotics. Those passive samplers enable further investigation for toxicological effects on the environment or populations of such mixture and concentrations. The polar C18 Chemcatcher has been evaluated for the monitoring of multi-families of Endocrine Disruptors and Pharmaceuticals. First C18 Chemcatcher with PES diffusion membrane has been calibrated for performance Reference Compound (PRC) approach. Performance Reference Compound (PRC) has been investigated [1]. Samples have been exposed for 28 days in a 50L flow through microcosm for the adsorption study and spiked samplers with tap water for the desorption experiment. Finally samplers were exposed for 3, 7, and 14 days in surface water in two rivers near Lyon. All 34 calibrated molecules showed good correlation to first order accumulation with half time of equilibrium above 14 days for most of those molecules. Good linear relationships were obtained (R² from 0.90 to 0.99) for 7 to 21 days on the C18 Chemcatcher. Sampling rates were calculated between 0.005 and 0.16 L·m³ day−1. Molecules showed accumulation and desorption consistent with first order kinetics with similar half time of equilibrium and thus verifying all criteria for their use as PRCs. Exposure campaign was validated by comparing TWA concentrations obtained from passive samplers to average concentrations detected in grab sampling analyzed by online SPE-LC-MS/MS method.

References

MO 013 Comparative analysis of pesticide monitoring via traditional surface water sampling and Chemcatcher passive samplers
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Pesticide monitoring in European surface waters is regulated by the Water Framework Directive (WFD) establishing environmental quality standards (EQS) for the good chemical status of water bodies. EQS monitoring is based on analyses of pesticide in water samples. WFD requires at least monthly sampling for assessment of maximum annual average concentrations. Due to the high temporal variation of pesticide use and leaching and discharge conditions in running waters, such robust monitoring approach may fail to detect pesticides, especially peak concentrations. We compared traditional water sampling results to pesticide concentrations detected with Chemcatcher® passive samplers in a large peatland river Kyrönjoki and its small tributary Lehmäjoki. The Chemcatcher® were deployed in four separate trials on both sites for two weeks time period during 21 Jun - 30 Aug. The traditional water samples were analysed twice a month in Lehmäjoki and monthly in Kyröjoki.

The number of compounds found in Chemcatcher® was 32, while in water samples 27 pesticides were detected but only 15 of them were quantified. High concentrations of some polar compounds were usually found with Chemcatcher® but not or at low levels with traditional grab sampling. While the Chemcatcher® was designed to detect polar and semivolatile compounds, traditional grab sampling techniques do not have the potential to eliminate this problem by providing time weighted average concentrations. Of the existing passive sampling technologies available, polar organic chemical integrative samplers (POCIS) show the most promise for assessing herbicide contamination and may also be used to measure concentrations of a wide variety of polar organic contaminants. In spring and summer 2010, POCIS were deployed in a site located through the South Nation River watershed, an agricultural watershed in Ontario, Canada. Three POCIS per site were deployed for two 28-day periods. One litre grab samples were taken at the beginning and end of each deployment to compare passive sampling with grab sampling. Grab samples were filtered, spiked with 1µg/L deuterated atrazine and contaminants concentrated on 500 mg Oasis® HLB cartridges. Extracts were analyzed for atrazine via LC/MSMS. Relative toxicity of the biological activity for each pesticide was calculated using an enzymic bioassay method. One issue with passive sampling in general is that daily sampling rates are influenced by a number of different factors such as temperature, stream velocity and biofouling. A calibration study to estimate how widely sampling rates vary in streams with different chemical and physical properties was conducted in fall 2010 and summer 2011 at six different streams. POCIS were spiked with 5oµg/L nicotine and propyzamide a atrazine (a metabolite with high fugacity). Both the fall and summer experiments were conducted over 28-days, with three POCIS removed from each field site weekly. Comparisons between grab sampling and passive sampling will be discussed, along with the feasibility of using deisopropyl atrazine as a performance reference compound. In addition, the merits of using passive sampling coupled with an algal bioassay to assess herbicide contamination and direct further monitoring will be discussed.

MO 014 Estimating herbicide contamination in flowing waters using passive sampling (POCIS) coupled with an algal bioassay
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Herbicide contamination of water bodies occurs mainly through run-off and leaching from agricultural fields as well as through spray drift. Traditional grab sampling is of limited value because this point of time estimation fails to account for sampling techniques in consecutive sampling pulses in continuous sampling regimes. We sampled 15 streams in Southern Ontario using the PRC approach to estimate temporal variation of herbicides. As PFOS have the potential to eliminate this problem by providing time weighted average concentrations. Of the existing passive sampling technologies available, polar organic chemical integrative samplers (POCIS) show the most promise for assessing herbicide contamination and may also be used to measure concentrations of a wide variety of polar organic contaminants. In spring and summer 2010, POCIS were deployed in a site located through the South Nation River watershed, an agricultural watershed in Ontario, Canada. Three POCIS per site were deployed for two 28-day periods. One litre grab samples were taken at the beginning and end of each deployment to compare passive sampling with grab sampling. Grab samples were filtered, spiked with 1µg/L deuterated atrazine and contaminants concentrated on 500 mg Oasis® HLB cartridges. Extracts were analyzed for atrazine via LC/MSMS. Relative toxicity of the biological activity for each pesticide was calculated using an enzymic bioassay method. One issue with passive sampling in general is that daily sampling rates are influenced by a number of different factors such as temperature, stream velocity and biofouling. A calibration study to estimate how widely sampling rates vary in streams with different chemical and physical properties was conducted in fall 2010 and summer 2011 at six different streams. POCIS were spiked with 5oµg/L nicotine and propyzamide a atrazine (a metabolite with high fugacity). Both the fall and summer experiments were conducted over 28-days, with three POCIS removed from each field site weekly. Comparisons between grab sampling and passive sampling will be discussed, along with the feasibility of using deisopropyl atrazine as a performance reference compound. In addition, the merits of using passive sampling coupled with an algal bioassay to assess herbicide contamination and direct further monitoring will be discussed.

MO 015 Performance of three passive samplers and sediment for detecting agrochemicals
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Due to intensive agricultural land use, Victorian waterways (South-East Australia) may contain a large number of agrochemicals (pesticides), some of which may prove toxic to organisms living therein, while others may elicit more subtle effects. Managing the effects of such contaminants ultimately requires information on efficient toxicity and chemical concentrations. Time integrative sampling of waterways with passive samplers (or passive sampling) is a technique which facilitates cost-effective monitoring of chemicals found at very low concentration in a range of aquatic ecosystems. In our presentation, we present the results of one of the most comprehensive examination of the input of pesticides in surface waters encompassing the measurement of 84 pesticides in sediments and three different passive sampling devices in 24 streams over a period of 5 month. Of the three passive samplers used, in short there were 28 pesticides observed amongst the 319 detections in the SBD-XC disk extracts. The most commonly observed chemicals (n=30) were simazine, mylobutanil, primicarb, pyrimethanil, imidacloprid, hexazinone, metalaxyl, atrazine and carbaryl. There were 27 individual pesticides observed amongst the 319 detections in the polar TRIMP samplers and 20 of the most commonly observed chemicals (n=10) being dieldrin, trifloxystrobin, pyrimethanil, and primicarb. Only seven agrochemicals were observed in extracts, although these observations included two chemicals not observed in the TRIMP or SBD-XC extracts, namely malathion and propyzamide. In natural sediments 34 pesticides were observed amongst a total of 266 detections, with the most commonly observed chemicals (n=10) being p,p'-DDE, simazine, mylobutanil, pyrimethanil, dieldrin and spinosad. This study confirmed that passive samplers appear most suited to investigation of non-polar compounds, with the Chemcatcher system utilizing an SBD-XC disk most suited for polar pesticides. Passive sampling provides a number of advantages such as the suitability of passive sampling to get a time weighted average concentrations. Of the 34 pesticides investigated, 13 toward specific compounds. Sediments collected a wide range of chemicals. Overall, sediments are probably most suitable to monitor a wide range of hydrophobic compounds, while passive samplers can complement important information on exposure kinetics, water concentrations and hydrophilic compounds.
Potential of different passive samplers to predict uptake of polycyclic aromatic hydrocarbons by crop plants

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Polycyclic aromatic hydrocarbons (PAHs) belong to a class of hydrophobic organic pollutants, produced during the incomplete combustion or pyrolysis of organic materials from primarily anthropogenic sources such as fossil fuels and agricultural waste. The fate of PAHs in the nature is of great concern to human health as these contaminants are widely distributed in the environment and are known to be carcinogenic.

The present study compares the ability of this suite of passive samplers to mimic uptake of 15 PAH prioritised by the European Scientific Committee on Food (ESCF) by crop plants grown in a hydroponic nutrient solution.

MO 021
Use of the semipermeable membrane devices (SPMD) as integrative tool for monitoring polycyclic aromatic hydrocarbons in tropical seawater


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To assess the capability of contamination monitoring in any system it is necessary to evaluate the concentration of these contaminants. It can be done using an organism surrogate, or biomimetic model, such as a passive sampling device as semi-permeable membrane devices (SPMDs). SPMDs are membranes composed of low density polyethylene. The present study compares the ability of this suite of passive samplers to mimic uptake of 15 PAH prioritised by the European Scientific Committee on Food (ESCF) by crop plants grown in a hydroponic nutrient solution.

MO 022
Development and application of a passive sampling technique for determining biomagnification factors and elimination half-lives in fish following dietary exposure

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Recent revisions to the OECD 305 test protocol have led to the introduction of a dietary exposure test in which the bioaccumulation potential of a substance is characterized in terms of its potential for biomagnification. The aim of this study was to test the hypothesis that the BCF and elimination rate Constants in fish tissue to that in the diet to which the fish is exposed at steady-state. Research was conducted to demonstrate the utility of passive sampling techniques for estimating BFs and half-lives of several hydrophobic chemical in fish tissue following an exposure in rainbow trout. In this study, five test substances (hexachlorobenzene, musk xylene, o-terphenyl, methoxychlor, benzo[a]pyrene) were used to assess dietary exposure in rainbow trout. The BMFs were determined for fish lipids and for fish tissues, respectively. The methodology involved determination of biomagnification factors and elimination half-lives for the test compounds in fish tissues following dietary exposure. The results were compared to those obtained using conventional dietary exposure techniques. The study showed that passive sampling techniques are a valuable tool for determining biomagnification factors and elimination half-lives of a wide range of contaminants in fish tissue following dietary exposure.
MO 023 Study of partitioning between lipid and passive sampling materials for equilibrium sampling P. Rusná, F. Smedes, H. Beebe1, J.A-N.A. Klanova1 Masaryk University, RECETOX, Brno, Czech Republic
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Sorption of organic phases like semi permeable membrane devices (SPMDs) as well as single phase polymer samplers (e.g. polydimethylsiloxane (PDMS), low density polyethylene (LDPE)) are widely used for monitoring of hydrophobic contaminants in the aqueous environment. Application of such passive samplers can be extended to monitoring of hydrophobic contaminants in fish tissue. Equilibrium concentration in PDMS sheets immersed in fish tissue can be easily converted to a concentration on lipid membrane. Using several types of passive samplers showed a good agreement with classical combustion analysis with classical analysis. So far, limited data on partition coefficients between lipid and passive sampler (Kips) are reported in literature. Kips can be calculated as the ratio of contaminant concentration in each phase at equilibrium. In the present work partitioning of major groups of hydrophobic contaminants as PCBs, PAHs, BDEs and various pesticides between several passive samplers (e.g. PDMS, LDPE) and various types of lipids (e.g. olive, fish, seal oil and a model lipid, trilinolein) is studied. The influence of temperature on the Kips is investigated. Equilibrium is confirmed by using performance reference compounds (PRCs) with properties equal to the properties of target compounds. Target compounds and PRCs are dosed to different media and an equal distribution of both compounds after equilibration demonstrates that equilibrium is achieved. Additionally diffusivity and solvent extraction material characterization by analysing the lipid patterns. The Kips values will contribute to implementation of monitoring in biota through passive sampling. Additionally when combined with existing water-LDPE partition coefficients, the obtained trilinolein-LDPE partition coefficients also allow to estimate water-SPMD partition coefficients for comparison with values presently applied in water passive sampling.

MO 024 Implementing WFD biota standards - a possible role for passive samplers? D.K. Ashton, P. Whitehouse1, A. Gravell2, B. Dordon1, B. Lyons1, J. Balassam1, D. Fraser1, P. Walker1 1Environment Agency, Wallnongford, United Kingdom 2National Laboratory Service, Environment Agency, Llanelli, United Kingdom 3Environmental Quality Standards Agency, Westminster, Ireland 4CEFAS, Weymouth, United Kingdom 5APEM, Oxford, United Kingdom

The UK’s rivers are at a requirement to monitor temporal trends of the levels of substances in biota and sediment. The use of biota for assessing compliance and trends presents a number of challenges, e.g. choice of appropriate species, availability of species at different sites, and the requirement of large numbers of fish or invertebrates to collect the required samples. We are not yet at a stage where we can implement biota standards with confidence. To address these concerns, we are exploring alternative ways for assessing nutrient concentrations in the same sites at the same time. Our poster describes our approach to determine the potential role of PSs as surrogates for biota. If quantifiable relationships can be discerned between chemical residues in biota, sediment and PSs, we may be able to use PSs as a surrogate for biota when assessing compliance with biota EQS. PSs and sediment samples have also been taken from the same locations. If quantifiable relationships can be discerned between chemical residues in biota, sediment and PSs, we may be able to use PSs as a surrogate for biota when assessing compliance with biota EQS. PSs and sediment samples have also been taken from the same locations. Analyses were conducted to determine the concentration of 13 compounds, including, HCB, HCBd, Pentachlorobenzene and TBT, in all four of the analysed sites. The results from the trial and the potential for using PSs for assessing compliance with biota standards and for trend analysis under the WFD will be presented.

MO 025 Measuring contaminant partitioning in Norwegian rivers I.J. Allan1, S.B. Rannekle1, C. Harman2 1NIVA, Oslo, Norway 2Norwegian Institute for Water Research, Oslo, Norway

Chemical monitoring under legislative structures such as the European Water Framework Directive (WFD) heavily relies on the application of environmental standards (EQS). Under the WFD, EQS values for priority organic substances have been set for the “whole water”. Conventional sampling-analytical methodologies fail to meet target performance parameters for WFD method selection. Passive sampling devices are able to provide improved limits of detection (when compared with bottle sampling) and in-stream contaminant measurements. However, concentrations measured with passive samplers are for the true dissolved fraction and do not include those bound to particle or dissolved organic matter (i.e. the “whole water”). Reconciliation between passive sampling measurement and EQS is needed. Derivation of EQS values for the truly dissolved phase is a long-term option. A shorter-term initiative may be to estimate river-specific suspended particle matter-water partitioning coefficients. These may be based on a simple assumption that the dissolved phase with passive samplers combined with techniques such as continuous flow centrifugation and in situ integrative SMPS samplers. In this study, we have measured the ability of semi-permeable membrane devices, silicone strips and low density polyethylene membranes dissolved phase concentrations of a range of non-polar priority substances in 6 river of the South East of Norway. These measurements were complemented by measurements in the suspended particulate fraction. Combining the two sets of results in a context with literature data on partition coefficients, a comparability of partition coefficients were estimated with repeated seasonal sampling in 2 of the rivers over a 2 year period. We propose here to derive estimates of whole water concentrations based on passive sampling measurements. Uncertainties associated with for example contaminants sorbed to dissolved organic carbon are discussed.

MO 026 Development of passive surrogate add-on devices for remote water sampling J. Hong, R.W. Williams, D. Janieson, C.A. Pohl Thermo Fisher Scientific (formerly Dionex, Sunnyvale, ca, United States of America

Most procedures for sampling remote bodies of water require the collection and shipment of the water samples from the site to the laboratory. In addition to the cost and inconvenience of collecting, bottling, preserving and shipping the water samples, there are potentialities for cross-contamination and errors at each step in the process. In another scenario, analytical instrumentation is available that is designed to be deployed in the field so that the analyses occur at-site. This second scenario for field testing is of course limited to the types of compounds supported by the instrumentation. A third scenario is the extraction of analytes from the water followed by shipment of sample-loaded concentration devices to a remote lab for analytical testing. We are most interested in this third scenario for the potentially lower cost and complexity and we report on our efforts to improve and perhaps validate data quality for this type of system by developing a passive surrogate addition device. A surrogate is a compound or analyte that is added to samples during preparation. The use of a surrogate is required in most sampling procedures that are approved for environmental testing in order to insure that the method is actually delivering data in the 70-130% recovery range. A surrogate generally has similar properties and responses in the analytical process to the target analytes and can be used to prove that the process is both capable and actually did deliver quality data. In remote sampling, there can be an issue with providing sufficient power to instrumentation including sampling pumps, valves etc. This can be easily solved by supplying a dual lithium battery power supply combined with consumption-monitoring electronics. If the system contains a mechanism for the addition of one or more method surrogates then one option is the use of a metering pump which of course would require power. In this poster we discuss our development of several passive systems for the addition of organic surrogates to sampling streams.

MO 027 Ambient thermal desorption ionization for rapid mass specrometry analysis of contaminants G. Bureau, M. Gillon, F. Zylberg, S. Guettard, D. Janieson, J.-M. Blanchard

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The use of a surrogate is required in most sampling procedures that are approved for environmental testing in order to insure that the method is actually delivering data in the 70-130% recovery range. A surrogate generally has similar properties and responses in the analytical process to the target analytes and can be used to prove that the process is both capable and actually did deliver quality data. In remote sampling, there can be an issue with providing sufficient power to instrumentation including sampling pumps, valves etc. This can be easily solved by supplying a dual lithium battery power supply combined with consumption-monitoring electronics. If the system contains a mechanism for the addition of one or more method surrogates then one option is the use of a metering pump which of course would require power. In this poster we discuss our development of several passive systems for the addition of organic surrogates to sampling streams.
Ultra low level determination of bisphenol A, 17β estradiol and poly aromatic hydrocarbons in river water using fully automated column-stitching HPLC

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Obtained Results reveal that MDI in alveolar fraction prevail the total collected particles, this can be allotted to the process used for cleaning and to the quality of formulated foam. In this work, we develop a rapid, reliable and low cost solution for the detection of organic micropollutants directly on site, based on multiple solid phases selective preconcentration (MSFP2) coupled to UV/Visible spectrometry and/or fluorimetry. The principle of the development is to combine several solid phase preconcentration cartridges (different chemical sorbents can be used) and various washing/elution solvents for the preparation and detection of many substances. A first approach have considered 13 substances among which pesticides, pharmaceutical products, PAH and endocrine disruptors in more or less complex mixtures. From the physico-chemical (pK_a, K_water) and optical (UV and fluorimetric spectra) properties of each target, and by mixing several sorbents and elution solvents, we tried to propose protocols for the detection of each component in the considered mixture. Such system can be characterised by its availability (rapid use and result in real time), simplicity (easy to transport and use by everybody), and rapidity (adapted procedure based on simple principle).

References:

HPLC analysis of isocyanate aerosol in workplace
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Workplace atmosphere is an important distribution of various pollutants, resulting from the handled products and concerned processes. The fate of these pollutants in the air (transport, deposition, degradation), is determined by their distribution between atmospheric particles and gases phase (Lohmann et al. 1998). Harmfulness of microparticles in workplace atmosphere and the diseases that they present are related to their chemical nature and size. The production of one kilogramme rigid polyurethane foam releases nearly 75% of total amount of polyurethane polyols and isocyanates in the air. In this work, we used the personal aerosol sampler CPI0-R (Cournier et al. 1998) and filters for evaluation of alveolar (Gorner et al. 1996) and inhalable fractions of particles during clean of casts after injection of the polyurethane foam (PU). HPLC was used for detection of MDI in different post.

Development and calibration of a passive sampler for N-nitrosodimethylamine (NDMA) in water

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N-Nitrosamines such as N-nitrosodimethylamine (NDMA) are organic compounds of contemporary interest in environmental waters, including groundwater, wastewater and potable water, due to their potent carcinogenicity in laboratory animal studies and probable human carcinogenicity. Heightened awareness of their properties and prevalence has led to increased scrutiny of water with Maximum Contaminant Level (MCL) goals ranging from 2 ng L-1 in California to 9 and 10 ng L-1 respectively in Canada and Australia and quantification limits down to low ng L-1 levels. USEPA Method 521 [1] for the analysis of N-nitrosamines in water is a coconut charcoal-based solid phase extraction method. This work investigated the use of the charcoal specified in this method as a passive sampler sorbent for NDMA and stipulates its use for other nitrosamines.

The suitability of coconut charcoal as a sorbent for NDMA has been previously investigated. Here we have designed pretreatment column[3]. This HPLC with backflushing system solved a recurrent issue of column clogging and the pretreatment column provided remarkable performance for removal of humic compounds from environmental samples. Use of this combination controlled ion suppression and increased peak response while using single stage MS as a detection system.

MO 028

Development of a communicating portable analytical system of organic micropollutants in water based on UV spectrometry-fluorimetry detection after multiple solid phases selective preconcentration
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Lots of organic contaminants such as regulated molecules (phytosanitary products, polycyclic aromatic hydrocarbon (PAH), [SDOTS]) as well as emerging pollutants (pharmaceutical products, endocrine disruptors, [SDOTS]) are generally present at trace level in environmental waters (in particular natural waters) except in case of accidental or intentional pollution where concentrations are stronger. In general, detection methods include, sampling, solid phase extraction (SPE) and finally gas or liquid chromatography-mass spectrometry (GC-MS or LC-MS) (Hilton et al. 2003, Mompilas et al. 2010). These protocols are necessary because standardized, but, they are limited when sudden pollution/contamination occurs and when rapid decision making is required. In this case, there is a growing interest to have available, rapid and on site detection techniques able to give information (qualitative, (semi)quantitative) of the type of pollution and the potential corresponding hazard for the environment and human health.

In this work, we develop a rapid, reliable and low cost solution for the detection of organic micropollutants directly on site, based on multiple solid phases selective preconcentration (MSFP2) coupled to UV/Visible spectrometry and/or fluorimetry. The principle of the development is to combine several solid phase preconcentration cartridges (different chemical sorbents can be used) and various washing/elution solvents for the preparation and detection of many substances. A first approach have considered 13 substances among which pesticides, pharmaceutical products, PAH and endocrine disruptors in more or less complex mixtures. From the physico-chemical (pK_a, K_water) and optical (UV and fluorimetric spectra) properties of each target, and by mixing several sorbents and elution solvents, we tried to propose protocols for the detection of each component in the considered mixture. Such system can be characterised by its availability (rapid use and result in real time), simplicity (easy to transport and use by everybody), and rapidity (adapted procedure based on simple principle).

References:
Mass balances and passive dosing of polychlorinated dibenzo-p-dioxins in a cell based bioassay
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Persistent organic pollutants are ubiquitous environmental contaminants and pose health risks. Cell-based bioassays can be cost-efficient and ethical alternatives to animal testing. However, the exact potency of the organic contaminants may be lower than that they really represent in natural systems due to biodegradation or growth medium and vials. In the present study we use the CALUX assay, which is specific for chemicals binding to the aryl hydrocarbon receptor and detects chemicals with a dioxin-like activity. For a series of polychlorinated dibenzo-p-dioxins we have assessed bioavailability and partitioning between medium components and cells and demonstrated how the potency fluctuates relative to pure water with more than 99% of the chemicals sorbed to medium and not volatilized from below 15% in the intensity of the assay. We also demonstrate how to overcome this limitation with a novel dosing technique where the polychlorinated dibenzo-p-dioxins are dosed from a polymer.

MO 034
Passive dosing as a tool to recreate the environmental mixture composition of HOCs in an aquatic toxicity test
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The aim of this study was to explore the potential of passive dosing for testing the toxicity of a PAH mixture that recreates the mixture composition found in seawater from a coastal area of Spain, the Bay of Algeciras. First, solvent spiking and passive dosing were compared for their suitability to determine the acute toxicity to A. franciscana nauplii of several PAHs at their respective solubility limits. Second, passive dosing was applied to recreate the seawater mixture composition of PAHs and to determine the limits of this approach at different levels. HPLC analysis was used to test the reproducibility of the dissolved exposures concentrations for the individual PAHs and mixtures. This study shows that passive dosing has some important benefits in comparison with solvent spiking for testing hydrophobic organic compounds (HOCs) in aquatic media. These include maintaining constant exposure concentrations, therefore higher reproducibility and a relative increase in toxicity. Passive dosing is also able to faithfully reproduce real mixtures of HOCs, such as PAHs, in toxicity tests, reproducing both the levels and proportions of the different safety factors. This provides a useful approach for studying the toxicity of environmental mixtures of HOCs, with a view to investigating their toxicity but also for increasing research on PAHs in mixtures result in different effects.

MO 035
Speciation of PAHs in stormwater
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Partitioning to particulate matter and dissolved organic carbon (DOC) has a large influence on the transport and fate of hydrophobic organic chemicals in aquatic environments. The application of hydrophobic organic compounds, called passive dosing, was used to measure partitioning of fluoranthene in samples of stormwater runoff. The freely dissolved concentration of 14C-fluoranthene in the samples was controlled by passive dosing from a pre-loaded polymer phase and the total sample concentration at equilibrium was measured. This method reveals the free fraction of fluoranthene in the sample as well as the enhanced capacity of the sample to carry fluoranthene relative to pure water. Free fractions of fluoranthene in the stormwater samples ranged from 0.15% in the first sample to 20-50% during the last part of the extensive experiment. Increased capacities of the stormwater samples for fluoranthene transport ranged from 2-23 relative to pure water. In the stormwater samples partitioning to the organic part of the suspended solids, measured by loss on ignition (LOI), dominated concerning partitioning to DOC. Results from different stormwater samples were taken at different events and at different times during the events, partitioning to LOI remains constant with KLOI = 154000 ± 3000 L/kg. These results can be used in stormwater quality modeling and assessment of the efficiency of stormwater treatment systems.

MO 036
Application of passive samplers for monitoring of cyanotoxins in drinking water
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Cyanotoxins including microcystins, so far they have been only rarely used in research and monitoring of cyanotoxins. Cyanobacteria and their toxins pose a threat to water quality and are of concern to water utilities. In order to establish seasonal and temporal trends, four long-range atmospheric transport and deposition (wet and dry) lead to a ubiquitous distribution of POP in the environment. POP accumulation in low temperature environments such as the polar regions and mountains is described as cold trapping. In addition, a fractionation of volatile organic compound mixtures can be seen toward the poles which is probably due to climate-controlled desorption, evaporation and re-deposition processes (“hopping”). It was hypothesized that especially at high altitudes of mountains remobilized contaminants may accumulate and contaminant patterns may change in order in the contaminants physic-chemical properties. In our study we used PAH and PCB as model compounds to study climate and altitude triggered fractionation processes in detail under field conditions. In a remote valley in the European Alps and in a valley in the Black Forest Mountains (Germany) test sites at various altitudes were equipped with chemical probes. These consisted of a porous ceramic tube (pore size ca. 5 mm) which were filled with various materials (quartz sand, peat and charcoal) to mimic different desorption scenarios from sorbents with varying sorption capacity. The materials were spiked with defined contents of PAH and PCB (20 μg/kg and 2 μg/kg) respectively. These probes were buried in soil in a maximum depth of 5 cm below surface. After several months the tubes were analyzed for evaporative PAH and PCB losses. After a total study period of 1 year, we found for quartz sand losses of volatile compounds such as naphthalene or phenanthrene close to 100% within the first 9 months at all altitudes. Fluoranthene showed a slight decrease of loss with increasing altitude which is in line with an expected fractionation. For stronger sorbents, e.g. charcoal and peat, losses were much smaller and for some tubes even an increase of the loading was observed. Higher-ring-PAH showed no loss or the results were within the analytical error. The PCBs with high volatility showed 100% loss after a 9 month period of deployment, while PCB with lower volatility showed a decreasing loss with increasing loss which could be confirmed from chemical probes analyzed after 12 month of deployment.

MO 037
Field evaluation of chemical probes to determine the fate of persistent organic pollutants in soil
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High-volume active samplers have been conventionally used for the environmental monitoring of persistent organic pollutants (POPs) in air. However, these systems have several disadvantages, such as high cost or power consumption, handling of reagents, and complex sample pretreatment. As an alternative, semi-quantitative assessments complementing active systems. Due to the low cost and easy handling, polymerurethane foam (PUF) disks are especially attractive. PUFS seem to be a useful tool to compare the state of pollution in specific areas, as well as for seasonal and spatial distributions of local POPs. In 2010, an environmental monitoring program of the municipal waste incineration plant of Vigo (Galicia, Spain) was initiated by monitoring PCBs and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs) and polychlorinated naphthalenes (PCNs) by means using passive samplers. In order to establish seasonal and temporal trends, for the campaign periods (March to November 2010 and January to September 2011) were carried out. PUFs were deployed for 3 months at 8 different locations around the facility. Levels of PCDD/Fs ranged from 0.087 to 0.038 pg WHO-TEQ/m^3, being very similar to those previously reported in the scientific literature. Regarding PCBs, the concentrations of dioxin-like compounds between 1.38 and 19.0 pg WHO-TEQ/m^3. The 7 environmental marker PCBs were noted in the airborne concentrations of POPs, the current levels of PCDD/Fs, PCBs, PCNs in air near MSWI are below or similar to those reported in other facilities in air. These results can be used in stormwater quality modeling and assessment of the efficiency of stormwater treatment systems. The research was supported by the SoMoPro project no. 25G2858 (funded from the European Community within the Seventh Framework Programme (FP/2007-2013) under Grant Agreement No. 229363 co-financed by the South Moravian Region).

MO 038
Seasonal and temporal trends of airborne POPs around a waste incineration plant by passive sampling methods
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High-volume active samplers have been conventionally used for the environmental monitoring of persistent organic pollutants (POPs) in air. However, these systems have several disadvantages, such as high cost or power consumption, handling of reagents, and complex sample pretreatment. As an alternative, semi-quantitative assessments complementing active systems. Due to the low cost and easy handling, polymerurethane foam (PUF) disks are especially attractive. PUFS seem to be a useful tool to compare the state of pollution in specific areas, as well as for seasonal and spatial distributions of local POPs. In 2010, an environmental monitoring program of the municipal waste incineration plant of Vigo (Galicia, Spain) was initiated by monitoring PCBs and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs) and polychlorinated naphthalenes (PCNs) by means using passive samplers. In order to establish seasonal and temporal trends, for the campaign periods (March to November 2010 and January to September 2011) were carried out. PUFs were deployed for 3 months at 8 different locations around the facility. Levels of PCDD/Fs ranged from 0.087 to 0.038 pg WHO-TEQ/m^3, being very similar to those previously reported in the scientific literature. Regarding PCBs, the concentrations of dioxin-like compounds between 1.38 and 19.0 pg WHO-TEQ/m^3. The 7 environmental marker PCBs were noted in the airborne concentrations of POPs, the current levels of PCDD/Fs, PCBs, PCNs in air near MSWI are below or similar to those reported in other facilities in air. These results can be used in stormwater quality modeling and assessment of the efficiency of stormwater treatment systems. The research was supported by the SoMoPro project no. 25G2858 (funded from the European Community within the Seventh Framework Programme (FP/2007-2013) under Grant Agreement No. 229363 co-financed by the South Moravian Region).
Can car cabin filters be a useful a sampling medium for air pollution monitoring purposes?

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This study focuses on the first time results of gas-chromatographic chemical analysis of car cabin air filters (CCAFs) collected from typical UK cars from the city of Manchester, United Kingdom. As a first step, this study focuses on polymeric hydrocarbon sorbents (PAHs), the most important category of semi volatile organic pollutants associated with traffic emissions.

Comparative study of PAHs in CCAFs ranged between 2760 and 10800 μg/liter, with benzo[a]pyrene, benzo[k]fluoranthene, benzo[ghi]perylene and chrysene being the most abundant PAHs. Benzo[a]pyrene (BaP) ranged between 315 and 740 μg/liter, accounting regularly for 5-15% of the total PAHs. Atmospheric BaP (and other PAHs) concentration were estimated by using different methods for ventilation rates, and duration of filter’s exposure and it was shown that the BaP levels of the air that CCAFs collect exceed 1000 ng/m3, a value of 1 ng/m3. Calculation of PAHs molecular diagnostic ratios shows the predominance of traffic related emissions.

We conclude that CCAFs are an invaluable alternative to conventional air sampling methods, featuring unique advantages, that are: a) the exposure estimation of car passengers (riders, pedestrian or road professionals), b) unlimited sample size, ideal for screening of the air quality, especially for compounds that react with particulate matter on the particles, c) provide, with an average, time weighted urban area air pollution assessment. The main disadvantage of this method is the uncertainty associated with accurate estimations of atmospheric pollutants concentrations, due to lack of knowledge of the exact amount of air filtered, and the fact that vehicles are used also for trips to less polluted places and the fact that also the recirculation of car cabin indoor air is undergoing filtration.

Observations on flame retardants (FRs) in indoor environments in Istanbul-Turkey

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Due to the several indoor sources of flame retardants-such as upholstery textiles and foam paddings in furniture and mattresses, thermoplastics for computers, TVs, electronic components and cables-indoor air is a very important exposure route of flame retardants (FRs). As they are less subject to photolysis and atmospheric dilution in indoor environments, they show increased air concentrations. Like outdoors, data of POPs indoors are sparse or lacking in Turkey. In this study we investigate PBDEs and non-PBDE flame retardants in houses, offices, electronic shops and upholstery-mattress shops indoors air and dust samples. The air samples were collected by using indoor air passive samplers. Dust samples were collected by using a 2 m2 area for 4 minutes. The main objectives of this study were a) utilization of polycyclic aromatic foam disc (PUF) passive sampling technique for indoor environments, b) to start the database on concentrations of flame retardants in indoor air and dust in Turkey, c) to investigate the congener profiles and partitioning of flame retardants in indoor environments, d) to evaluate flame retardants exposure through indoor air and dust pathway. For these purposes, the planned study is in progress and samples analysis is being carried out at the present.

Comparison of four modeling approaches to describe chemical uptake by passive air samplers

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Over the past decades, various passive air samplers (PASs) have been developed to monitor semivolatile organic compounds (SVOCs) in air. Uptake in PAS occurs by molecular diffusion from air to a passive sampling medium (PSM), such as polyethylene (PE), polyurethane coated glass (POG), polyurethane foam (PUF), and XAD-resin. Unlike in PUF or POG passive air samplers, where SVOCs accumulate in thin layers in contact with air, PUF and XAD are porous materials. The Whitman two-film model, which was originally developed to describe mass transfer from air to water, is often used to describe chemical transfer from air to the PSM. This model requires that "the mass of the liquid phase is continuous throughout the layer," whereas the mass transfer in the PSM occurs by thin, non-continuous films of liquid, such as PUF and XAD. Chemical transfer within such PSM only occurs in the gas-filled pores, which limits the transfer rate because only a low fraction of the SVOCs is in the gas phase. Recent experimental evidence confirms that SVOCs do not distribute uniformly within the PUF and XAD, which directly contradicts the assumptions of the Whitman two-film model.

In this study, we investigate the congener profiles and partitioning of flame retardants in indoor environments, a) utilization of polycyclic aromatic foam disc (PUF) passive sampling technique for indoor environments, b) to start the database on concentrations of flame retardants in indoor air and dust in Turkey, c) to investigate the congener profiles and partitioning of flame retardants in indoor environments, d) to evaluate flame retardants exposure through indoor air and dust pathway. For these purposes, the planned study is in progress and samples analysis is being carried out at the present.

Layer by layer self-assemble acetylcholinesterase and gold nanoparticles on glassy carbon electrode for sensing organophosphate pesticides

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Organophosphates(OPs) are highly toxic compounds to the vertebrates, which can be absorbed readily through the skin, mucous membranes, gastrointestinal and respiratory tract. With the increasing awareness of environmental protection and health concerns, there is a growing public concern about the accumulation of OPs in foods products and water supplies.

An sensitive amperometric biosensor for the determination of OPs on Layer-by-layer (LBL) self-assembled acetylcholinesterase (AChE) and gold nanoparticles(AuNPs) on glassy carbon electrode(GCE) is described. The GCE is modified with AuNPs by electrostatic interaction between AuNPs and cationic poly(diallyldimethylammonium chloride) (PDDA). AChE is immobilized on the negatively charged AuNPs surface by alternatively assembling a PDDA layer and an AChE layer.

F. Kang, R. Xue

Layer by layer self-assemble acetylcholinesterase and gold nanoparticles on glassy carbon electrode for sensing organophosphate pesticides

A novel approach to addressing metal and metal nanomaterial bioavailability in soils

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Earthworm influence on metals bioavailability in relation with metal speciation and ecotoxicity

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Metal-enriched atmospheric fine and ultra-fine particulate matters (PM) are currently rejected in environment increasing significantly ecosystem pollution by metals. Few data are available concerning their impact on human health. In the context of Reach regulation, the study of pollutant fatigue and impact on the biosphere is required, as well as efficient tools development for sanitary risk assessment. Earthworm is a key organism to assess soil quality. By its bioturbation activities, earthworms could modify metal bioaccessibility and bioavailability in soils (in relation with metal localization and speciation). In this context, several experiments are performed to: (i) Estimate the difference of metals bioaccessibility and bioavailability between polluted soils influence by bioturbation or not; (ii) Determine earthworm influence on metal speciation by studying ingested soils and casts; (iii) Assess ecotoxicology impact of metal from atmospheric PM on earthworms.
- Different PM and polluted soils (historically polluted and spiked soils); two species of earthworms: L. terrestris (ecologically relevant) and E. fetida (international standard for ecotoxicology tests) will be used for laboratory or field experiments. For the different soils before and after bioturbation: i) metal bioavailability will be determined by in vitro UBM test; ii) metal phytoavailability will be assessed by the study of plant uptake (lettuce); iii) microscopic (MIE-EDS) and spectrometric (Raman, XRF et Exafs) techniques will be used to perform elementary maps and determine potential changes in metal speciation (cast study); iv) general parameter like survival, biomass changes, cast production and burrowing activities will be studied to assess metal ecotoxicity.

- Strong influence of particle size and metal speciation on metal bioaccessibility was observed; variation between 20 and 82% according to experimental conditions. Moreover significant ecotoxicity effect on earthworm (using cast-production and biomass change measures) was highlighted.

MO 046
Lead bioavailability to soil invertebrates as affected by soil physicochemical characteristics
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- The bioavailability and toxicity of lead to terrestrial invertebrates may be markedly varied by variations in the physicochemical properties of soil. Other factors that can affect the bioavailability of Pb include aging and weathering of contaminated soils. The results of this study confirm the toxicity tests most conducted with soils freshly amended with metal salts and the relevance of data from such tests to contaminated field soils is unclear. This study compares the chronic toxicity of Pb to Eisenia fetida and Folsomia candida in seven European soils varying widely in physicochemical properties (i.e., pH, organic matter content, texture, cEC). Toxicity across all seven soils was compared using soils that were aged for 4 and 2 months, respectively.

- The results of the exposure of Eisenia fetida to Pb-contaminated soils showed that the bioavailability of Pb to E. fetida was low and not affected by the soil properties. Leaching and aging generally attenuated the toxicity of Pb to both soil invertebrates studied. The toxicity data, together with leaching and aging correction factors, provide important information on the bioavailability of lead to invertebrates in soil under realistic conditions.

MO 047
Lead bioavailability and bioaccessibility in sediments from wetlands with different hunting management in Tuscany (Italy)
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- Lead bioavailability and bioaccessibility in sediments from wetlands with different hunting management were investigated. In this study, we investigate the relative bioavailability and bioaccessibility of Pb in sediments from two Italian wetlands “Diaccia-Botrona” and “Padule di Fucecchio” (inland marsh) where previous studies showed high Pb levels. Sediment was sampled from areas with different degree of Pb shot pellet densities in each wetland, including hunting ranges and hunting estates and nature reserves. The relative Pb bioavailability was evaluated using the Community Bureau of Reference (BCR) sequential extraction procedure, unlike the relative Pb bioavailability was estimated using an "in vitro" simulation of the gizzard-intestine system. Pb partitioning obtained with BCR procedure showed a similar pattern for “Diaccia-Botrona” and “Padule di Fucecchio”. Percentage of total Pb concentration in sediment was less than 6% in the fraction 1 "acid extractable" with exchangeable and carbonate bound metal, about 72-80% in the fraction 2 " reducible" with iron manganese oxide/hydroxide associated metal, about 4.25-16% in the fraction 3 "oxidizable" with metal bound to sulfide and organic matter, and about 3.5-18.6% in the fraction 4 "residual" with metal in the mineral phase.

- Despite of the similar pattern of BCR partitioning of Pb in the two wetlands, results of "in vitro" simulation showed percentages of bioaccessible Pb in gizzard phase (pH 1.5) two order of magnitude higher for “Padule di Fucecchio” than for “Diaccia-Botrona” in both the shooting ranges (14% and 0.18%, respectively) and natural reserve areas (10% and 0.10%, respectively).

- Due to the non-acidic conditions of the intestinal phase (pH 6.5), final Pb bioavailability were below of detection limits in both study areas. According to these results, direct ingestion of Pb shot pellets can represent a higher risk for birds than the unintentional ingestion sediment contaminated by the disaggregated pellets in heavily hunted areas.

EMO2P1 - Fate and exposure modelling

MO 049
Evaluation of a molecular fragment-based tool for predicting pp-LFER descriptors of complex and multifunctional organic compounds
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- Poly-parameter linear free energy relationships (pp-LFERs) are useful models to accurately describe equilibrium partitioning processes of neutral organic chemicals in technical and natural systems. However, only a limited number of pp-LFER descriptors are available so far for complex and multifunctional compounds as well as for environmentally relevant substances. This hampers the application of pp-LFER models for estimating environmental partitioning behaviour of pollutants. The experimental determination of these descriptors is tedious and time consuming. Especially for screening purposes, the ability to predict solute descriptors just based on the molecular structure would be highly desirable.

- The commercially available software ABSOLV is an easily accessible and manageable tool that predicts solute descriptors based on a SMILES input. Here we evaluate the performance of ABSOLV to predict partition coefficients using calibrated pp-LFER equations in combination with predicted ABSOLV descriptors. This evaluation is done for various partition processes and a large set of complex, multifunctional organic solutes.

MO 050
Model suite to estimate the air/water partition coefficient of organic compounds at 25°C
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2Inter-Institute for Environmental Chemistry, University of Vienna, Austria

- The new model suite named E is one of the very properties in environmental partitioning. A number of estimation models for E are available from literature. However, despite of the recently published new experimental data, at least to our knowledge there was no relevant new model published within the last years. The poster presents a critical review of the existing models through validation with a huge up-to-date data set. Furthermore, this set has been employed to develop three new models based on fragments, Abraham parameters, and read-through at atom-centred fragments.

- The performance of the new models is presented, and the inter-connection of the model errors for the different new and literature approaches is examined. Bundling all methods in a model suite thus allows for a sophisticated consensus modelling of E. All referred and new models are implemented and publicly available in the public free edition of ChemProp (see http://www.ufz.de/index.php?en=6738 for details).

SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
The development of these models was partly supported from the EU Integrated Project OSIRIS (contract No. 037017).

MO 051
ChemProp - chemical properties estimation software system
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MO 052
The concentration of pollutants lasting in the intake through inhalation by humans. Deposition fluxes in soil, sediment and water determine environmental exposure which of course also indirectly affects humans. In this poster, the exposure of water and air will be mainly focused due to deposition processes. Dry and wet depositions from the atmosphere are estimated using the 2004 version of the ECE/CTOC TRA (Targeted Risk Assessment) integrated tool based on the Ops model parameter estimation for the calculation of the chemical concentrations in environmental media due to atmospheric deposition. Persistence of the model such as the source description, the emission height and the heat content of the emission plume, suggested by de Bruin et al. (2010). Deposition fluxes in the soil compartment will be calculated on the basis of these refined model parameters and discussed.

MO 053
Establishing a postgraduate course on exposure modeling for environmental risk assessment
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2UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany
3University of Oslo, Oslo, Norway

MO 054
Parameter reliability in kinetic evaluation of environmental metabolism data - Assessment and the influence of model specification
J. Ranke1, R. Lehmann2
1University of Osnabrueck, Osnabrueck, Germany
2Radboud University, Nijmegen, Netherlands

MO 055
Development, application, and evaluation of models for screening organic chemical exposures to humans
J.A. Arnott1, T.N. Brown1, K. Brevik1, F. Wanati1, M.S. Mcclachlan1
1University of Toronto Scarborough, Toronto, Canada

MO 056
World exposure assessment tool
J.M. Cheplick, A.M. Ritter, W.M. Williams
Waterborne Environmental, Inc., Leesburg, United States of America

MO 057
Uncertainty analysis using a Fugacity-based multimedia mass balance model: application of the updated EQC Model to DS
J. Kim
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Among several multimedia models, the Equilibrium Criterion (EQC) fugacity multimedia model has been widely used for the assessment of the fate, distribution and transport...
of a chemical of interest in the environment. Since the model requires partitioning and reactive properties of the chemical as input data, it is very important to understand how much uncertainties of the physicochemical properties affect the model outcomes. Thus, this study focused on the uncertainty analysis of the EQC Level III model for decamethylcyclopentasiloxane (D5) to quantify the confident range of mass distribution, persistence and intermedia transport rates based on pre-determined dispersion factors, transport properties, and reactivities of the chemical. For the uncertainty analysis, the Monte Carlo simulation technique was employed using Oracle Crystal Ball as an Excel Add-in program to a new spreadsheet platform of the EQC model. The uncertainty analysis showed that once it is released or vaporized to air D5 stays in the compartment until it is removed at a relatively fast rate via OH radical oxidation and adsorption to aerosol. Since D5 is not meant to be a semivolatile compound and nonintermedia transport from air to other compartments. These model outcomes are not very affected by the uncertainties of physicochemical properties of D5. On the contrary, when D5 is released to water, a major fraction is distributed in sediment due to sorption of the compound to organic substance where the degradation rate is very small. In this case, the model outcome is predominantly influenced by the uncertainty of KOC. Under a worst-case scenario (94.5% to air, 0.8% to water and 4.7% to soil), the 95%-confident-range of the mass distribution in air and sediment are 47%-78% and 19%–49%, respectively, whereas the overall persistence varies from 3.2 to 5.8 days with the same confidence. The variances are predominantly affected by KOC. Overall, the EQC model results are strongly dependent on the mode of emission. Since the mass distribution in sediment is sensitive to the emission to water, accurate measurement of KOC and Kd is expected to warrant a better understanding of the fate, distribution and transport of D5 in the environment.

MO 059
Prediction of environmental and biological degradation half-lives with quantitative structure activity relationships
T.N. Brown, J.A. Arnott
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A review of existing models shows that currently published and operational models - ranging from single medium high resolution transport models to low resolution multimedia multi-pathway models are not able to properly reflect local to global impacts of emissions of pollutants, nor are they flexible enough to be adapted to specific locations or grid geometries. We already demonstrated innovative features of a new model called Pangea, aiming at addressing these needs, in a single-source context based on emissions of various types of plants located in the north-east of France. This model provides a statistical result based on the study of more than 500 power plants located around the World, simultaneously demonstrating the flexibility of Pangea. A set of multi-scale multimedia modeling with the relevant media and refined around each source (coal power plants) are build. These grids allow Pangea to evaluate and compare local to global impacts of each source using a standard multimedia fate/transport and multi-pathway population exposure approach, but spatialized specifically for this study. Substances taken into account were chosen to represent broad categories of known environmental pollutants.

Results obtained so far for a few sources indicate that for volatile pollutants (e.g. Benzene) less than 10% of the overall population intake might happen within a 10km radius from the source, and that a local modeling (e.g. local risk assessment) of such emission might capture only a small fraction of the overall impact. We will present a statistical results on the comparison of local to global evolution of the integrated intake fraction. In other words, we will discuss statistically the fraction of the global intake that is captured by a local modeling, for a distribution of coal power plants (at their true locations) that covers the globe.

MO 060
Partitioning of organochlorines in storage lipids, membrane lipids and proteins in lean fish
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Lipids are the dominant force in determining organic contaminant accumulation in aquatic organisms because hydrophobic organic chemicals (HOCs) are believed to partition mainly into lipid phase. Therefore, lipid normalized tissue concentrations have been used to reduce variation in tissue residues between different species and aid in understanding contaminant toxicity and distribution between media. However, partitioning of contaminants between different lipid classes is not very well known. Lipid composition contributes to variation in bioaccumulation potential and neutral storage lipids are thought to be the most important lipid class for HOCs. On the other hand, polar membrane lipids are the target for many HOCs toxicity and knowing the concentration in membrane lipids would greatly improve the risk assessment of HOCs. Using a PLS analysis as a partitioning phase, we have applied equilibrium partitioning approach to estimate HOC distribution in storage lipid, membrane lipid and protein fractions for carbon 14 labelled PCB-153, PCB-77 and PCB. The method is tested with the extracted native PCB contaminated pikeperch oil, and measures of lipid class and protein contents in fish. PCB distribution was dominated by storage lipids (70-78%) while membrane lipids (19-23%) and protein (2-6%) had lower share. The results showed the importance of chemical partitioning to protein fraction in lean fish. The results give new important insight of estimating toxicity using chemical body burden as a dose metric.

MO 061
Simulating ichonogenic chemical fate, bioaccumulation and exposure with RAIDAR
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RAIDAR (Assessment, Identification And Ranking) model includes linked fugacity-based multimedia fate and bioaccumulation sub-models, thus providing the capacity to evaluate exposures to humans and ecological receptors in a "holistic" manner. The general objective of this project is to revise and evaluate the RAIDAR model for the simulation of ionogenic chemicals for screening level endpoint predictions of risk and uncertainty. The model may still be used under data-poor conditions. This presentation outlines the model revisions and explores the fate, bioaccumulation and exposure potential of ionogenic chemicals released to the environment. A hypothetical set of ionogenic chemicals are used to simulate how changes in chemical partitioning properties and dissociation constants affect chemical distribution and risk to different receptors of interest (i.e. fish, terrestrial vegetation, bird). Simulations are also conducted for current ichogenic chemical data and exposure potential.

MO 062
Predicting the oral uptake efficiency of chemicals in mammals: extension to the hydrophobic range
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Environmental risk assessment requires models to estimate bioaccumulation of untested compounds. So far, most mechanistic bioaccumulation models have focused on very lipophilic compounds, and only few included also more hydrophobic compounds. As shown by pharmaceutical studies, the membrane permeation of polar compounds is not sufficient due to the low octanol water partition coefficient (KOW). Additional descriptors are needed to mechanistically estimate membrane permeation. Our aim was to develop a model to estimate oral uptake efficiency of pollutants via ingestion in mammals for polar and nonpolar compounds over a wide KOW range of pollutants with focus on polar compounds. The model was based on mechanistic processes and thus included the desorption process of the pollutant through the gastrointestinal tract, the diffusion from the food through the unstirred water layer, through the membrane and into the blood. The diffusion through the membrane was split into an inner and outer membrane resistance, where KOW reflected partitioning from the water into the outer membrane, and hydrogen bond donor strength reflected the
partitioning from the outer into the inner membrane. The model was calibrated to two data sets which were merged: uptake efficiency of environmental pollutants measured in different mammals during feeding studies, and a pharmaceutical data set with human oral absorption efficiencies.

Comparing mass transfer of PAH from soil to clothing, CWA extraction from ACU (and DD), and Contact Transfer of CWA from soil onto clothing, A. Rehn et al. (2012) showed that the quantity of CWA transferred from soil surfaces contaminated at operationally inconsistent in compositions, of CWA, have been reliably established for some CWA and ongoing testing continues for others; but need still existed for a reliable transfer-exposure method for assessing the exposure potential for chemicals sorbed onto soil. We have established a method for reliably determining Contact Transfer of compounds from soil directly onto clothing, utilizing sorption of compounds as a standard procedure to produce transferable concentrations to be integrated into the model equations. The results with the experimental data, still a deeper understanding of the processes determining the chemical fate of PAH in the system of seawater, algae and mussels is required.

Despite promising microbial degradation of polyaromatic hydrocarbons (PAH) in the laboratory, pilot-scale investigations and observations at the field scale often reveal the formation of a considerable residual fraction of PAHs, which is not further degraded. This residual fraction may be due to adsorption and sequestration which may increase with time. However, residual concentrations may also be due to kinetic reasons, i.e., the slow decline of PAH concentration may be due to the formation and persistence of a metabolite in the inner membrane. The model was calibrated to two data sets which were merged: uptake efficiency of environmental pollutants measured in different mammals during feeding studies, and a pharmaceutical data set with human oral absorption efficiencies. In the present study, we tested the hypothesis that the initial bacterial numbers were high enough to achieve complete mineralisation within the experiment duration. The observed variability and to predict future trends, a new dynamic modelling approach (DynANet) based on an existing water-sediment model (DynA) was applied. In DynANet, the mass balance of polycyclic synthetic musks using a new dynamic segmented surface water/soil model was described using a three compartment model. First order kinetic equations were used for the description of accumulation and depuration of selected PAHs in mussel tissues. Data were obtained from experiments performed with selected PAHs under constant conditions. In the model, three compartments were defined: (1) mussels, (2) surrounding environment (seawater), and (3) algae (Phaeodactylum tricornutum) as food source for the mussels. During the bioaccumulation period, the mussels take up compounds both from seawater and with PAH contaminated algae. Thus, the model considers dynamic exchange of PAHs between algae and seawater. Experimental data were used to parametrise and calibrate the modelled soil (and DD) with inorganic mussels was started. Samples of water and suspended solids were collected seasonally in 7 monitoring stations located up- and downstream of the 3 sewage treatment plants (STPs) present along the river. Seasonality in concentrations was observed, with the highest values in the autumn and winter months. More structured samples collected downstream of the 3 STPs showed the highest variability and to predict future trends, a new dynamic modelling approach (DynANet) based on an existing water-sediment model (DynA) was applied. In DynANet, the implementation of geographic information system (GIS) tools allows the subdivision of a river drainage basin in sub-basins and the resulting river links, classified according to the Strahler stream order, communicate by means of a downstream flow. The loading data of runoff water and modelled chemical concentration coming from the different links are then routed to the outflow of each link, in such a way that the concentrations of a chemical in the downstream link are the sum of the concentrations of the chemical in the upstream links, plus the concentration transferred from the soil compartments and working in a GIS environment. The application of the DynANet model to the case study allowed to compile a preliminary mass balance for HHCB and AHTN and to predict concentration changes under environmental phenomena such as precipitations events, temperature variations, soil use, etc. The measured concentrations and simulated results were compared to the prediction of existing models such as GREATER.
MO 068

Estimating chemical emissions from home and personal care products in Asia
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Asia's economy is growing significantly with China and India leading the growth. This has led to an increased demand for home and personal care (HPC) products. Information on the production, use and fate of HPC products is essential in estimating emissions and predicted concentrations (PECs). It has also been demonstrated that by coupling population density data with sales data it is possible to estimate HPC product consumption. To date, most studies have been based in developed markets, where wealth distributions are more homogeneous and HPC products are affordable to all resulting in usage being evenly distributed across the population. In Asia, a population's wealth and ability to buy HPC products is not uniformly distributed, therefore combining information on population density and sales data with a sub-population’s ability to purchase HPC products could improve chemical emission estimates. Global datasets were sourced for population and economic activity incorporating population, night-time light satellite imagery and land cover. Coupling these datasets with sales data of different HPC products at a (sub) country level and 'takeoff values', (a population ability to purchase HPC products based on its per capita GDP), emission estimates were calculated. Results show large variation in HPC product usage exist across Asia due to the variability in population density and wealth. The largest refinement of emission estimates are associated with products requiring greater purchasing power. As wealth e.g., for example the per capita GDP is above $10,000, population density becomes the appropriate method for distributing product use. The potential benefits of this approach include the accurate estimation of chemical emission patterns, which could be used to parameterise exposure models for use in environmental risk assessment and contextualising existing monitoring data.

MO 069

Chemical fate and risk assessment at large scale based on direct and inverse modeling approaches and pan-European datasets
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The chemical fate and risk assessment at large scale is typically performed on the basis of explicit GIS fate models which in turn make use of either a “direct” or an “inverse” modeling approach with the direct being a traditional one. The direct models are based on a priori available information about chemical emissions and are intended to answer the question: “Where do chemicals go after being emitted?”. Thus, when data for emissions and substance’s physico-chemical properties could be retrieved from existing databases, the model predictions of spatial distribution of chemicals prove to be sufficiently accurate for screening purposes.

Oppositely, the inverse modeling deals with the question “where do pollutants come from?” and is based on measurements of chemical concentrations. Then, the inverse models support large scale assessment, in particular for the surface water quality by estimating chemical emission factors at regional, river basin or continental scale in relation to the population density or another appropriate proxy.

This work presents the potential of the European version of MAPPE (Multimedia Assessment of Pollutant Pathways in Environment) direct model to simulate a steady-state spreading of pollutants emitted from point or diffuse sources. The model outcome consists of annual fate maps of chemical concentrations, deposition rates, fluxes to other environmental compartments, and concentrations used for screening of hazard zones at continental scale with spatial resolution 1 km2. In this model exercise the target contaminants were few POPs as PCBs, dioxins, furans, pesticides, etc.

In addition the study encouraged the applicability of the inverse modeling technique for pollutants with poor and sparse emission estimates but which were extensively monitored in the environment. An example of the application of the inverse models was utilised for backward tracking of PFOA and PFOA emission factors from riverine measurements and to investigate the loading of the widely used pharmaceuticals and personal care products to European rivers.

MO 070

Multimedia assessment of chemical exposure by MAPPE-Global explicit model: a case study of pollutant discharge to Mediterranean Sea
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The work describes a case study of pollutant discharge to Mediterranean Sea by a spatially explicit fate and exposure model with worldwide coverage for Multimedia Assessment of Pollutant Pathways in the Environment (MAPPE Global). The model computes removal rates of a substance with given physico-chemical properties in an evaluative environment composed of atmosphere, soil, inland and sea water, in the form of global maps with a spatial resolution of 1°x1° for air and oceans and 0.08°x0.08° for inland water surfaces.

The impact of including ice and snow on model-predicted distribution and fate was studied using the global multicompartment chemistry-transport model MAPPE Global, a coupled atmosphere-land-ice-ocean model. The model was used to study the environmental fate and transport of γ-HCH and DDT, with the the results suggesting that the inclusion of ice and snow compartments improves model performance. The results suggest that the effectiveness of emission control measures may significantly vary among substances, not only reflected as latitudinal gradients, but also as longitudinal gradients.

MO 071

The impact of including ice and snow in the multimodal modelling of persistent organic pollutants’ distributions and fate
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Persistent organic pollutants (POPs) are multimodal substances. Ice or snow compartments have only recently been included in POP cycling in large scale multimodal modelling.

The impact of including ice and snow on model-predicted distribution and fate was studied using the global multimodal chemistry-transport model MAPPE Global, a coupled atmosphere-land-ice-ocean model. The model was used to study the environmental fate and transport of γ-HCH and DDT, with the results suggesting that the inclusion of ice and snow compartments improves model performance. The results suggest that the effectiveness of emission control measures may significantly vary among substances, not only reflected as latitudinal gradients, but also as longitudinal gradients.

MO 072

Global contamination of PCBs
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PCBs are ubiquitous environmental pollutants which are expected to decline in abiotic environmental media in response to decreasing primary emissions since the 1970s. A coupled atmosphere-ocean general circulation model with embedded dynamic sub-models for atmospheric aerosols and the marine biogeochemistry and air-surface exchange processes to soils, vegetation and the cryosphere [1-3] is used to study the transport and fate of four congeners (3-7 Cl atoms) 1950-2010. The global distribution of the PCBs evolves over time reflecting sources and sinks’ evolution over time. Globally, secondary emissions are on the long term increasingly gaining importance over primary emissions. They are most important for congeners of medium hydrophobicity (5-6 Cl atoms). Congeners’ fractionation is characterized both geographically and temporally. It causes enrichment of the lighter congeners and more delayed decreasing levels in high latitudes in response to decreasing emissions. Delivery of contaminants to high latitudes is predicted to be more efficient than previously suggested.


Predicting the removal of atmospheric particles by vegetation with a dynamic multimedia model
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It is well known that association of capturing contaminants from air. In particular, plant leaves can act as a biological filter and as a sink for airborne particles and their associated Semi-Volatile Organic Compounds (SVOCs) such as Polycyclic Aromatic Hydrocarbons (PAHs), influencing their environmental fate. In order to assess the filtration performance of plants with respect to atmospheric particulate matter (PM) and their associated SVOCs, deposition on vegetation has recently been investigated by a number of different methods (field, laboratory studies as well as modelling approaches). Some authors studied the particulate matter uptake and retention capacity of different plant species, showing that some plant type are much more efficient than others at collecting particulate matter and PAHs contained upon them. Other researches were focused on investigating the fate of air pollution velocities not only in the urban tree canopy (not necessarily the vegetation compartment) but also at different wind speeds. Furthermore different predictive approaches were developed to evaluate PM uptake by trees in urban areas and to estimate dry particulate matter deposition on leaf surfaces using species-specific deposition velocity. Nevertheless, the existing multimedia fate models do not generally account for PM mass balance in predicting PM deposition to vegetation and the corresponding chemical flux. In the present study we developed such a fate model concept, which incorporates such vegetation compartment, to dynamically assess and compare the influences which are caused by the uncertainty in chemical property data and variability in climate variables. Representative polychlorinated biphenyl (PCB) congeners having a range of physical-chemical properties are used to test the hypothesis that property uncertainty has more influence on model outcomes than climate variability. The robust statistical tool Oracle® Crystal Ball in chemical property data and variability in climate variables. The purpose of this study is to explicitly assess and compare the influences which are caused by the uncertainty in chemical property data and variability in climate variables. Representative polychlorinated biphenyl (PCB) congeners having a range of physical-chemical properties are used to test the hypothesis that property uncertainty has more influence on model outcomes than climate variability. The robust statistical tool Oracle® Crystal Ball (Fusion edition, 11.1.1.0.0) is adopted to perform the correlation and sensitivity analysis. Generally speaking, results suggest that uncertainty in physical-chemical property data has more pronounced variances in the model predictions than variability in climate variables, i.e., the predicted environmental fate and distribution of selected PCB congeners is more sensitive to uncertain physical-chemical properties than to climate variables. The uncertainty analyses with Monte Carlo simulation determined that hydrolysate half-life in water and sediment layer depth was the most influential input parameter to dynamically assess the short-term air-canopy exchange and the influence of biological, climatic and land cover drivers on the canopy capacity of accumulating pollutants from the atmosphere. The chemical fate model accounts for effects of short-term variations in air temperature, wind speed, stomatal opening and leaf energy balance, all as a function of layer in the canopy. The model also includes a new wet deposition scheme that estimates the interception of gases dissolved in rain as a function of depth in the canopy, and accounts for the potential occurrence of intense short-medium term re-emission showed (UCD) to up to 10.7 fold variation as a result of environmental forcing. In addition, relatively small inter-annual variations in seasonally-averaged air temperature, canopy biomass and precipitation can produce relevant changes in the canopy storage capacity for the chemicals, suggesting that climate change may play a significant role in determining the effectiveness of forests in controlling atmospheric transport of semivolatile pollutants.

Assessing and comparing the influences of uncertainty in chemical property data and variability in climate variables on the simulated fate of PCBs
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Logestic pollutant inputs (POPs) are of concern due to their persistence, bioaccumulation potential, toxicity and ubiquitous presence in the natural environment. Environmental fate modelers have made numerous efforts to simulate the transport and fate of legacy POPs at different spatial and temporal scales. The success of modelling exercises depends largely on the quality of model input data characterizing either the modelled chemicals or the modelled environment, e.g. the physical-chemical properties and environmental (including climate) variables. The purpose of this study is to explicitly assess and compare the influences which are caused by the uncertainty in chemical property data and variability in climate variables. Representative polychlorinated biphenyl (PCB) congeners having a range of physical-chemical properties are used to test the hypothesis that property uncertainty has more influence on model outcomes than climate variability. The robust statistical tool Oracle® Crystal Ball (Fusion edition, 11.1.1.0.0) is adopted to perform the correlation and sensitivity analysis. Generally speaking, results suggest that uncertainty in physical-chemical property data has more pronounced variances in the model predictions than variability in climate variables, i.e., the predicted environmental fate and distribution of selected PCB congeners is more sensitive to uncertain physical-chemical properties than to climate variables. The uncertainty analyses with Monte Carlo simulation determined that hydrolysate half-life in water and sediment layer depth was the most influential input parameter to dynamically assess the short-term air-canopy exchange and the influence of biological, climatic and land cover drivers on the canopy capacity of accumulating pollutants from the atmosphere. The chemical fate model accounts for effects of short-term variations in air temperature, wind speed, stomatal opening and leaf energy balance, all as a function of layer in the canopy. The model also includes a new wet deposition scheme that estimates the interception of gases dissolved in rain as a function of depth in the canopy, and accounts for the potential occurrence of intense short-medium term re-emission showed (UCD) to up to 10.7 fold variation as a result of environmental forcing. In addition, relatively small inter-annual variations in seasonally-averaged air temperature, canopy biomass and precipitation can produce relevant changes in the canopy storage capacity for the chemicals, suggesting that climate change may play a significant role in determining the effectiveness of forests in controlling atmospheric transport of semivolatile pollutants.

Environmental fate, latitudinal distribution and long range transport of decamethylcyclopentasiloxane (D5) in the global environment: a model assessment
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Decamethylcyclopentasiloxane (D5) is widely used as an ingredient in the formulation of personal care products, or is present as an impurity in the silicone fluids and rubbers. The global environmental fate, latitudinal distribution, and long range transport of D5 was analyzed by two multimedia chemical fate models using the best available physicochemical properties as inputs and known persistent organic pollutants (POPs) as reference. The global transport and accumulation characteristics of D5 differ from those of typical POPs in three significant ways. First, a large fraction of the released D5 tends to become airborne and is removed from the global environment by degradation in air at a rate that has a tendency to be distributed and persistent in all media. Secondly, although D5 can travel a substantial distance in the atmosphere, it has little potential for deposition to surface media in regions. Compared with a deposition potential of known POPs that exceeds that of D5 by at least 4 orders of magnitude. Thirdly, D5 has a short global residence time with the majority of the global mass removed within three months of the end of release. Global residence times of POPs on the other hand are in years.

Environmental fate of D5 Predicted by the QWASI Fugacity model
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Due to the unique characteristic property of decamethylcyclopentasiloxane (D5), airborne D5 remains in the compartment and influence of OH radicals. In contrast, when D5 is released to water, multiple processes including volatilization, sorption to sediment, advection and hydrolysis take place competitively. Importantly, the rates of transport and transformation processes are dependent on the local aquatic environment. Since a better quantitative assessment on the fate, distribution and transport of D5 in local water body system is needed, this study evaluated the environmental behavior of D5 in Lake Ontario (LO) and Lake Pepin (LP) at various environmental temperatures and under steady-state and unsteady-state conditions. Additionally, uncertainty analyses were performed to determine the most probable range of the model outcome and the most influential input parameters using a new Quantitative Water Air Sediment Interaction (QWASI)-Excel model. The QWASI-Excel model predicted that water concentration of D5 decreased for both lakes due to increase in hydrolysate rate with rising temperatures from 1 to 2.5°C. In contrast, D5 concentration profiles in sediment were different: it decreased gradually from 1.0 to 0.5 mg/l in LO whereas it increased from 120 to 280 mg/l in LP. D5 mass preferentially distributed to water in LO but to sediment in LP. Mass fraction of D5 increased with temperature for both lakes mainly due to increased KOC value. D5 was hydrolysed at higher temperature via adsorption. A significant increase in concentration was predicted for both lakes whereas residence time of D5 in sediment was much longer due to slow degradation. In contrast with a relatively short response time in water, response time in sediment was relatively long. Uncertainty analyses with Monte Carlo simulation determined that hydrolysate half-life in water and sediment layer depth was the most influential input parameter to environmental behaviors of D5 in LO and LP. In addition, other factors such as KOC, hydrolysate half-life, solid deposition rate and sediment resuspension rate also contributed to the total variations in environmental behaviors of D5 in LP. The analysis also predicted 95% confidence intervals for D5 concentrations in water and sediment, mass distribution, intermedia transport and transformation rates, residence time and response time in both lakes.
A study of the relevance of regionalization and archetype approach for aquatic freshwater ecotoxicity

MO 079

The model shows lower estimates of transport from source to receptor than previous studies, which lacked a precipitation module. Uncertainty in input data and modeling assumptions, which will be discussed, allow the model to be applied to large-scale systems as a comparative tool. For example, the ingested intake fraction for aluminum emissions varies between 3% and 10% of the total for a food chain of 1x10^6 or more, depending on the location. The continuum of emission, via its hydrology and the fraction of treated/untreated drinking water, plays an important role.


MO 080

Mathematical modeling of point source pollutants fate and transport in the Little Akaki River, Ethiopia

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Computer models are becoming increasingly important tools in every environmental management aspects. A general one-dimensional water quality model has been developed for one of the most industrially concentrated and polluted rivers in Ethiopia - the Little Akaki River. The study covers a total of 27.4 km length along the river where pollution issues have been reported. The spatial and seasonal dynamics of the river hydro-geometry in a power law model. For this purpose, 20 locations were systematically selected and field data were collected for five months. The river has been conceptualized as 1.57 equal length completely mixed computational elements, and a functional representation that includes mass transport and hydrodynamic equations were used for each computational elements. The approach used the numerical technique by finite volume spatial discretization and finite difference temporal discretization. Computational solution procedures were prepared by writing a computer program code using the MATLAB programming platform (version 7.0). BOD and DO were selected as water quality indicators, and sensitive model parameters that affect fate and transport of DO and BOD in the river were determined. The calibration and validation of the calibrated model in predicting data not used during calibration was also estimated and the result was in good agreement with the targeted water quality data. The applicability of this model as decision making tool for pollution control activities was also assessed and the result is encouraging.

Recommendation, which takes into account the existing water quality problem in the study area, has been suggested for improvement and wider application the model.

MO 081

A study of the relevance of regionalization and archetype approach for aquatic freshwater ecotoxicity

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The importance of regionalization for the aquatic freshwater ecotoxicity impact category has been evaluated at different resolutions (at a watershed, country and continental scale) using multimedia fate and exposure models such as IMPACT World, Europe single zone and Europe spatial models.

A first comparison of fate factors between the IMPACT Europe single zone model vs. the IMPACT Europe spatial model has been performed considering both a uniform emissions and emissions in each specific watershed. Moreover three watersheds with short, medium and high residence time of water to the sea have been selected to analyze the importance of chemical properties vs. watershed specific properties to identify the key parameter(s) influencing the fate. The water residence time was used as the basis to split the watersheds into three different types: i.e. upstream with water residence time >0.1 [yr] and downstream of a lake (water residence time <0.1 [yr]). The fate factors of chemicals emitted into water calculated with this latter approach have been compared to the spatial model and differences with the a-spatial approach discussed.

The results of IMPACT World and Europe showed that a-spatial models might overestimate the chemical fate and characterization factors for fresh water ecotoxicity up to a factor of 5 compared to a spatially differentiated model for unknown emission location (i.e. assumed being uniformly emitted compared over the whole model surface).

When the emission location is known, a spatially differentiated model can improve the model accuracy up to 2-3 orders of magnitude, because of its ability to accurately predict the water residence time to the sea (or out of the system) depending on the emission location. Is therefore spatial differentiation always required? The answer depends on the properties of the chemical: only for persistent chemicals the water residence time plays a key role in determining the chemical fate for freshwater ecosystem. Highly degradable or volatile chemicals for example would disappear before being advected out of the system. This support that, for persistent chemicals, the country/ regional differentiation is relevant.

MO 082

Mixing zones modeling in the water framework directive: comparison between “discharge test” and "CORMIX". M. Eliot, P.A.J. Van Straten, ARCHE, Gent, Belgium

The Priority substance Directive 2008/105/EC has defined EQS for 33 priority substances. Compliance with these EQS should be achieved at the point of discharge or at least at the location of the receptor in the receiving water body. Consideration has now been developed to assess whether compliance with the EQS values is achieved. The approach starts with simple investigations but could end with very detailed assessments on the mixing behavior and concentration profile of effluents in the surface waters. In contrast to lower tiers, higher tiers require an estimate of the extent of EQS exceedence that can be used to define a mixing zone. This may be achieved using a numerical approach for each computational elements. The aim of this presentation is to discuss the application range of both “Discharge Test” and “CORMIX”. "Discharge Test" is a freely available model developed to assess the acceptability of mixing zones resulting from discharges into surface waters at Tier 2 level. This model is based on Fischer equations and requires a limited set of parameters and allows for quick modeling of effluent concentrations from single port submerged pipes only. "CORMIX" is a USEPA supported model that can be used for Tier 2 and higher. It is a mixing zone expert system that classifies momentum and buoyancy of the discharge in relation to boundary interactions to accurately predict different mixing behaviors. “CORMIX” is a well-validated model that requires just a few more input parameters. CORMIX takes into account all the weaknesses pointed out for “Discharge Test”. "CORMIX" was designed to present less worst case results, it was designed to provide the most accurate representation of the actual physical mixing process given a set of ambient and discharge conditions. The main conclusion is that “Discharge Test” is easy and fast and can be used for Tier 2 assessments of single port submerged discharges. If more accurate modeling is required or if “Discharge Test” does not cover the discharge parameters, “CORMIX” could be used.
Over the years, the GREAT-ER model has served a worldwide user community, and currently more than 170 different scientific papers refer to the development or application of the model.

Under the sponsorship of the CEFIC-LRI programme, the package has now been updated again as GREAT-ER 3.0 (2011). Most notably, the database has been replaced by the Open Water Quality Database system (OWQDS). For the first time, the entire system can be operated without any software license fee, and the scientific community is welcome to analyze and Free Software GREAT-ER. On the model side, GREAT-ER 3.0 now also includes a sediment extension, and the possibility to model lakes as part of a river basin.

Information on GREAT-ER and updates on different projects initiatives can be found at www.great-er.org.

MO 084

Case study using the tiered approach for mixing zone modeling in the water framework directive

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The Priority Substance Directive 2008/105/EC has defined EQSs for 33 priority substances. Compliance with these EQSs should be achieved at the point of discharge or at least at the edge of the mixing zone in the receiving waters. Considering this, a “Tiered Approach” has been developed to assess whether compliance with the EQS values is achieved. The approach starts with simple investigations but could end with very detailed assessments on the mixing behavior and concentration profile of effluents in the surface water. The Tiered Approach starts with estimations of the extent of EQS exceedence to lower tiers, higher tiers require an estimate of the impact of the effluent on the aquatic environment. The Tiered Approach enables to define a mixing zone. This may be achieved using a range of tools such as “Discharge Test” or “CORMIX”. The aim of this poster is to present a test case using the tiered approach. An industrial site has a surface discharge with a flow rate of 1,000 m³/d to a small river characterized by a flow rate of 18,000 m³/d. Concentration of the contaminant of concern in the effluent [CoC] is 35 µg/L which is below the EQS of the CoC. If an EQS is present in the effluent and the [CoC] > EQS therefore triggering the initiation of the second tier. The Process Contribution (PC) is 2.63 µg/L representing 13% of the EQS after complete mixing. This is higher than the allowed 4% and therefore higher tiers should be initiated and an estimation of the extent of the mixing zone is required. Surface discharges are best modeled with CORMIX requiring detailed knowledge of discharge characteristics, effluent, and receiving water. Due to the importance of the near field interactions, both the geometry and position of the discharge has to be well defined. In this case study, the discharge is located on the left bank point perpendicular to the river with a local depth at discharge of 0.4 m and a slope of 5 degree. The discharge channel is 0.3 m wide and 0.3 m deep. CORMIX simulations show that at approximately 0.6 m down stream of the discharge point the [CoC] drops below the EQS. A proposed extent of the mixing zone is described in the extent of EQS exceedence of 10⁻⁶ m, which in the test case would be 20 m. Therefore the [CoC] will be below the EQS in the proposed mixing zone of 20 m and compliance is achieved.

MO 085

Source apportionment of chemicals under the WFD

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New European Parliamentary Standards (EQS) are being set under the Water Framework Directive (WFD) for a wide range of substances. Generally, the concentrations for these EQS are lower than existing values. However, the sources of many of these substances are wide ranging, particularly for metals and nutrients, with multiple diffuse and point source inputs into the aquatic environment. Where an EQS is exceeded, Programmes of Measures (PoMs) may be required to reduce inputs to the aquatic environment subject to considerations of technical infeasibility and disproportionate costs. The WFD states that compliance should, as a priority, be achieved via source control, applying the “polluter pays principle”. To apply effective PoMs it is therefore necessary to accurately quantify sources of chemicals discharged to the environment. A review of available methodologies demonstrates that there are no available national scale source apportionment models. UK Water Industry Research (UKWIR) in collaboration with the Environment Agency and SEPA funded the development of a model for future river basin planning. The project comprised a number of key tasks: (1) specification and securing of national datasets, (2) generation of databases for different sector inputs (point and diffuse), (3) upgrading of the SIMCART water quality model for monthly outputs and in-river partitioning for metals, and (4) the development of a GIS-Simcat interface for data processing and results visualisation.

The project allowed prediction of loads of copper, nickel, lead, cadmium, mercury, zinc, PAHs, nitrogen, phosphorus and DEHP to 1 km2 resolution for urban runoff, highway runoff, sewage effluent, storm tank discharges, combined sewer overflows, agricultural runoff from livestock and arable land, atmospheric deposition, septic tanks, mine water discharges, natural background erosion and industrial inputs. A series of detailed catchment scenario investigations were carried out on the Wear, Tame and Hampshire Avon catchments in the Midlands and south of England respectively to assess the impacts of diffuse and point source phosphorus inputs in order to develop PoMs that may be considered as part of the second cycle of river basin planning.

MO 086

Persistence of petroleum hydrocarbons in seawater: modeling versus experimental results

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Hydrocarbons are a highly reduced form of carbon which provides a valuable source of energy for microorganisms. Therefore, mechanisms have evolved to degrade them, and nearly all hydrocarbons can be degraded under appropriate conditions. However, degradation of petroleum hydrocarbons in biodegradation studies is often underestimated, mainly due to the intrinsic physicochemical properties of these substances. One of these properties is their low water solubility, which results in low bioavailability to microorganisms. Biodegradation data are required by many regulatory frameworks around the world. In the persistence assessment for petroleum substances under the EU REACH regulation, biodegradation data for the major hydrocarbon classes present in these substances were used. In order to screen for persistence, aquatic half-life predictions for representative hydrocarbons were calculated using the BioHCwin module of the EPISuite v4.0 model. These predictions allowed narrowing the range of potentially persistent hydrocarbon classes and carbon ranges, thereby optimizing biodegradation testing efforts. To experimentally test biodegradation for the hydrocarbons of interest, a new experimental methodology was used which determined single hydrocarbon biodegradation in seawater. To prevent variability due to water solubility constraints, a passive dosing system was used to load the seawater with the chosen hydrocarbons. Biodegradation was measured using a respirometer, which performed automated dissolved oxygen measurements in the test vessels and minimized variability due to experimental handling. Except for highly branched structures or structures with quaternary carbon, experimental results showed that in general, seawater biodegradation model predictions were overly conservative. It can be concluded that the combination of modeling results and targeted experimental data provided a complete and robust assessment of the persistence of petroleum hydrocarbons.

MO 087

Field to continental phosphorus fate and eutrophication modelling

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To date, it has been difficult to link agriculture practices with local, regional, and long-distance impacts. Using a milk-producing farm as an example, this work demonstrates the possibility for improved environmental impact analysis based on the integration of site-specific biogeochemical models, local hydrology data, and fate and impact models, linking from the local to the regional scale.

An integrated model (Manure-DNDC) [1] was extended to include phosphorus (P), and a new, global P transport model was developed. Based on site-specific information, the Manure-DNDC model calculates total phosphorus loss from the milk producing farm. Subsequently, watershed specific data is used to estimate the amount of P that reaches the local stream. Once it is delivered to the stream, is routed downstream using a newly developed approach [2] that incorporates advective transport, deposition in waterways due to physical settling and biological uptake, and removal from the water system due to water withdrawal. The environmental impact of these incremental phosphorus additions can be assessed by comparison to existing phosphorus concentrations.

Manure-DNDC was used to simulate the runoff flow, sediment yield, and P loads for two test farms. Predictions showed agreement with validation data within a factor of two, and always within one order of magnitude. The model predictions of the fate of emitted phosphorus show that total ecosystem impact, and the location of that impact, is strongly dependent on the location of emission.

In the case of emission to the Great Lakes, the long residence time of water means that the majority of impacts can occur within 200 km of the point of emission, depending on whether impacts are assumed to occur at all existing P concentrations or whether there is a threshold value. For emissions in the Mississippi watershed, ~25% of impacts occur within 50 km; ~45% occur between 50 and 200 km, and ~30% occur greater than 200 km away.


MO 088

ENM fate in freshwater through adaption of USEtox (tm)

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Engineered nanomaterials (ENMs) have in recent years received substantial attention, both in scientific and consumer circles, as these materials are introduced to a steadily increasing number of consumer products. This has led to environmental concerns on how this novel material class behaves in the environment, at which concentrations

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organisms are exposed to the materials and what effects these materials may have on the environment. In relation to metal-oxide engineered nanomaterials (ENMs), as is the general case for ENMs, many environmental aspects are still unknown and/or hence not properly scientifically mapped. One approach that has not been given much attention in relation to environmental assessment of ENMs, more precisely the fate, exposure and effect modelling of metal-oxide ENMs is the application of adapted characterization models designed for single (chemical) compound assessment e.g. the USEtoxTM model for characterisation of ENM effect potentials.

The purpose of this study is to evaluate if existing characterisation model such as the USEtoxTM model can be applied for characterisation modelling of ENMs applying adapted ACM. The primarily goal of this study is to propose an adapted ACM for the characterisation of ENMs. The characterisation was done by relating nano-material properties to chemical properties and hence model the nano-material as a chemical with representative fate and exposure patterns. In the case study involatile ENMs' (metals) were characterised in USEtoxTM applying adapted characterisation modelling. The result obtained indicates that with some limitations the approach is considered valid - the characterisation factors are considered uncertain relating to several facts such as lack of environmental studies on ENMs making is hard to assess the general environmental behaviour of ENMs and hence relate this environmental behaviour to similar "chemical behaviour".

MO 089

Use of multiple tracers to assess non-stationarity of hydrologic transport at the catchment scale

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This study, in the use of multiple conservative tracers is proposed in order to calculate the arrival time distributions of a solute through hydrologic transport. The variability of the heterogeneity of the soil-water conditions (soil properties, preferential flowpaths, evapotranspiration losses, etc.) acting as a stochastic hydrologic filter suggest non-stationary solute travel time distributions. Several travel-time based models have already been developed, but experimental validation is still lacking for this type of models. In order to establish experimental travel-time distributions, 1) temporally displaced tracer injections and 2) spatially displaced tracer injections are conducted in the hillslope of the Chamberone river (Lusasone, Switzerland). Tracer concentrations are measured downstream both close from the injection site and at the catchment outlet in lake Geneva. In the first experiment, pulse loads of different tracers are injected at the same spot near the stream, in order to emphasize the effect of antecedent soilwater conditions resulting from climatic forcing and hydrologic filtering. The extent of the soil water deficit is the main trigger of non-stationarity in the arrival-time distributions. In the second case study temporally displaced injections are conducted. The algorithm offers the option to represent multi-channel river systems, using a looped mesh, and artificial structures such as dams. The solute transport is computed under similar soil-water conditions resulting from the advection-diffusion equation, using the ADIS code coupled with the hydraulic model. This code permits to take interactions between chemicals into account . Finally, the transport of particulate contaminants is simulated with the help of erosion/deposition laws (TS code). Thus, sediment stocks and erosion areas can be located, which is of huge interest for the identification of contaminant accumulation areas. This numerical model can predict the fate of chemicals resulting from accidental contamination, as well as chronic contamination. In the first case study, passive tracing data (Rhodamin WT) were used to identify the longitudinal dispersion coefficient and to verify the results of the model. In the second case study focused on suspended sediment and particulate contaminants (PCBs and Hg) transport, based on data collected during dam flushing operations conducted on the upper Rhone River. Two case studies demonstrated that the numerical model proposed can be an effective tool for determining concentration, transport and fate of contaminant in the aquatic environment (solid and liquid phases), offering a robust base for environmental exposure assessment studies.

MO 091

Emissions of anthropogenic pollutants in the Alpine areas: the GEMINA project

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Emissions of anthropogenic pollutants are estimated in the Alpine area using a reliable estimation of river concentrations of contaminants in the environment. In aquatic systems, contaminants are transported by water (liquid phase) as well as by suspended particulate matters (solid phase), complicating the determination of their occurrence, source and fate. We introduce here an efficient numerical model for the prediction of contaminant transport in a river network and present results of two case studies for both dissolved and particulate contaminants. The hydraulic conditions are simulated using a 1D hydrodynamic model (MAGE code), which presents very low computational times and allows for long-time simulation at a regional scale. The algorithm offers the option to represent multi-channel river systems, using a looped mesh, and artificial structures such as dams. The solute transport is computed under similar soil-water conditions resulting from climatic forcing and hydrologic filtering. The extent of the soil water deficit is the main trigger of non-stationarity in the arrival-time distributions. This numerical model can predict the fate of chemicals resulting from accidental contamination, as well as chronic contamination. In the first case study, passive tracing data (Rhodamin WT) were used to identify the longitudinal dispersion coefficient and to verify the results of the model. In the second case study focused on suspended sediment and particulate contaminants (PCBs and Hg) transport, based on data collected during dam flushing operations conducted on the upper Rhone River. Two case studies demonstrated that the numerical model proposed can be an effective tool for determining concentration, transport and fate of contaminant in the aquatic environment (solid and liquid phases), offering a robust base for environmental exposure assessment studies.

MO 092

Bioaccessibility options for detailed quantitative risk assessment of metals

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The Characterisation of Specific Geochemical processes (CSG) and the Common Assessment Criteria (CAC) have been developed to enable Generic Quantitative Risk Assessment (GQRA). One of the main assumptions in the derivation of these characterisation criteria is that 100% of the contaminant present in the soil will be bioavailable or bioaccessible. Although this assumption may be necessary in the preliminary stages of risk assessment to ensure the protection of human receptors, it may result in overly conservative risk assessment. Recommendations for remediation may subsequently be made in situations where remediation is not appropriate.

Recent years the potential for bioaccessibility data to be incorporated into Detailed Quantitative Risk Assessment (DQRA) has been increasingly recognised. To investigate the potential for the application of bioaccessibility data, a review of the CAC and GQRA criteria was conducted, including a characterisation of the Contaminated Land Exposure Assessment model (CLEA). GAC25 have been derived for selected metals; arsenic, cadmium, mercury, nickel and vanadium for standard residential, commercial and allotment land uses. By comparing GAC25 to typical contaminant concentrations encountered in UK soils we explore the options for the future application of bioaccessibility data in DQRA.

A risk evaluation of the need to remediate based on the use of literature estimates of bioavailability or of GAC that invoke generic bioavailability values would be difficult to defend. GAC25 however may be helpful in deciding whether to incur the cost of site specific studies of bioavailability. The applicability of GAC25 can be determined by comparing the existing evidence: history of contaminant formation (natural or anthropogenic); geochemical sequential extraction and physiological based tests. The natural or anthropogenic geochemical processes that led to the formation and current distribution of the contaminants of concern (CoC) at a specific site will give an indication of the likely chemical instability and therefore bioavailability of such CoC. Sequential extraction testing can give an indication of the mineral species with which different fractions of the CoC are associated and hence their likely availability. Finally empirical physiologically based tests can give an insight into the material specific chemistry.

MO 093

Urban versus rural GIS-based distinction for human health impacts in LCA. Application on automotive fuels

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Life cycle assessment (LCA) is frequently used to assess the sustainability of the various alternative fuels that are emerging. However, local impacts, especially human health (HH), are often neglected. This is because they are heavily dependent on the local context and thus difficult to understand. In this study, we introduce two distinctions in order to improve HH impacts in LCA of alternative fuels : urban versus rural and high versus near-ground emissions.
We assess the HH impacts associated with volatile organic compounds (VOC), particulate matter, nitrogen oxides and sulphur dioxide atmospheric emissions (main pollutants emitted by cars). To assess their impacts, two indicators are retained: USEtox (for VOCs) and ReCiPe2008 particulate matter formation potential (PMF, for the other pollutants). Two systems are studied: internal combustion engine (ICE) cars (using gasoline and diesel fuel) and electric vehicles (represented here by a Renault Fluence ZE using power coming from hard coal and natural gas).

To assess the rural and urban parts of atmospheric emissions, a geographical information system (GIS) was developed, allowing knowing the share of urban emissions all along the life cycle of the systems studied. It is then coupled with USEtox distinction between urban and regional emissions. For PMF, the ReCiPe methodology was coupled with the ExternalE project, allowing differentiating between urban and rural emissions.

For distinction between near-ground and high emissions, the same methodology using Ecosense was applied to PMF. For USEtox, the characteristic of the compartments, especially the mixed height of the air compartment, were modified. These values are then taken from the results of the previous study, applying a rural/urban distinction has a strong effect on USEtox and PMF results. Secondly, the high/near-ground distinction also modifies the results, especially for ICE cars. Finally, the superiority of one fuel on another is complex to assess, as we did not study all HH impacts (heavy metals emissions and photochemical ozone formation are not assessed here). Further research should focus on the following topics: extension of the methodology to photochemical ozone creation, extension to the whole car LCA, integration of the results with other impacts and assessment of damages (endpoint level).

MO 094
A new version of input decision for the selection of modelling endpoints for PECgw simulations for harmonisation of exposure assessment

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The EU and national registration processes require the assessment of the leaching potential of an active ingredient and its metabolite(s) of plant protection products (PPP) to groundwater as described in the FOCUS Groundwater report (2000 and 2009). For groundwater simulations using FOCUS-PELMO 4.4.3 in the framework of the national registration procedure in Germany new recommendations related to Tier 1 and Tier 2 were published by the Federal Environment Agency (UBA) and the German Agrochemical Industry Association (IVA) (Holdt et al. 2011). Input Decision 3.1, an easy to handle MS Excel-tool, was developed to select sorption endpoints for realistic worst case groundwater modelling concerning a relationship between sorption behaviour and soil properties e.g. organic carbon content (OC), pH-value or cation exchange capacity (CEC). Furthermore, a procedure has been developed to select degradation endpoints depending on a significant correlation between DT50-values and soil pH-value as well as taking the variability of DT50-values into account. The significance of a correlation between degradation and/or sorption behaviour and soil properties is investigated by the Kendall rank correlation test that is implemented in this tool, too. This method represents a reliable statistical test when only a few values (e.g. min. 3 to 6) are available.

Input Decision 3.1 provides:

- Clearly arranged templates for normalised laboratory DT50-values depending on temperature and moisture of soil degradation studies using either default or measured values of soil moisture and/or temperature,
- Statistical evaluation of dependency between DT50-values and pH-values from laboratory soil degradation studies and recommendations for the selection of DT50-values and the scenario,
- Statistical evaluation of dependencies between Kf/Koc and soil properties and recommendations for Kf/Koc and scenario selection.

Both procedures are the simultaneous result of several correlations between sorption and degradation endpoints as well as for the Kendall rank correlation test and the derivation of modelling endpoints for DT50 and Kf/Koc-values linked to the recommendation to apply one or two suitable scenario(s) in FOCUS-PELMO 4.4.3 simulation relevant for the German groundwater assessment. The approach presented here may contribute to a harmonisation of exposure assessment for PPP, between national, zonal and European level in future.

MO 095
Estimation of soil moisture and temperature based on different soil hydraulic pedotransfer functions

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The temperature normalisation procedure as described by FOCUS (2006) can be used to normalise soil field dissipation rates and to derive DT50 value standardised to 20°C and pH = 2. This normalisation procedure decreases or increases individual day lengths by means of daily correction factors based on soil moisture and soil temperature. Hence, soil properties such as moisture and temperature also need to be available in daily resolution. For many soil dissipation studies in the field, these parameters are, however, not readily available, at least to still run a realistic assessment, average daily soil moisture and temperature values can be estimated with predictive models, e.g. PEARL. For the present assessment, van Genuchten parameters are derived from the soil properties of a trial site located in Central Europe. Therefore, three different types of soil hydraulic pedotransfer functions are tested. The respective results will be compared to measured soil conditions to determine a simulation method that represents realistic field conditions, e.g. climate data, soil capillary pressure, global temperature, global precipitation rates. Additionally, measured soil moisture (10 cm depth) and soil temperature values (5 cm and 20 cm depth) for 12 data points were available, respectively. Hydraulic pedotransfer functions were prepared based on the HYDRUS database (Nemes et al. 2001), the Rosseta database (USDRA 2000) as well as the Stirling Series (Wosten et al. 1994, Van Genuchten 1980). For each approach a specific PEARL scenario was created using the corresponding van Genuchten parameters. For the PEARL simulations a soil profile of 1 m in depth were defined. The profile consisted of five horizons (20 cm each). Soil characteristics were only available for the uppermost horizon (0-20 cm) and they were duplicated to be identical in all horizons. The PEARL soil moisture and soil temperature simulations were evaluated for the first horizon (0-20 cm) for different depths steps (1 cm resolution). The simulated daily soil moisture and soil temperature data from the respective depths were compared to soil moisture and soil temperature measurements from sampling sites. Using a goodness-of-fit indicator (sum of squared errors between simulated and estimated volumetric soil moisture), the estimation method that represents measured data from the trial site the best will be determined.

MO 096
Bank filtration simulation model SIMBaFi - a tool to refine the PECgroundwater as part of the environmental risk assessment of medicinal products for human use

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Model calculations are an important component for the exposure estimation of human pharmaceuticals within their environmental risk assessment following the EMA guideline (EMEA/CHMP/SWP/44470/06, June 2006). In Phase II Tier A of this guideline the potential entry of a pharmaceutical into the groundwater (PECgroundwater) by bank filtration is calculated by multiplying the predicted environmental concentration of surface water (PECwatersurface) by a factor of 0.25. This factor does not consider substance specific properties, or originated by expert judgment. The mathematical simulation model SIMBaFi was developed in a project to replace the multiplier (0.25) by a model based decision matrix taking into account the following parameters: distance between shore-line and extraction well, depth of filter screen, hydraulic conductivity and average extraction rates. The result was a Microsoft Access based tool that is capable to simulate the fate of active substances in the soil environment. Microorganisms occur in soil pores either in suspension or as biofilms which alter the pore geometry and the activity field of microorganisms degrading those substances depends on soil structure. The model is applied to investigate the degradation behaviour of pesticides in a range of soils through variation of the degree of heterogeneity. This underlines the significance of the effect of soil structure and microbial activity field on transport and degradation of pesticides.

MO 097
Biodegraders in heterogeneous soils: reactive transport of active substances revisited

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The heterogeneity in soils is influenced by its texture, density, porosity, and the distribution and state of microbial life. Soil physical properties as well as microbial life have an impact on the fate of active substances in the soil environment. Microorganisms occur in soil pores either in suspension or as biofilms which alter the pore geometry and the activity field of microorganisms degrading those substances depends on soil structure. We will present an upscaled model which combines spatial stochastic generation of soil structures at pore scale and a process model at column scale coupling fluid flow, reactive transport, and biofilm dynamics. Process models are formulated by a system of coupled partial differential equations which is solved by a Petrov Galerkin scheme (COMSOL Multiphysics); the spatial stochastic process model is based on Gaussian Random Fields (R). The model is applied to simulate the fate of a pesticide (sum of squared errors between simulated and estimated volumetric soil moisture), the estimation method that represents measured data from the trial site the best will be determined.

MO 098
LCA studies of biofuels in multi-output bioeconomies

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The holonic nature of Life Cycle Assessment (LCA) is becoming an indispensable feature in the assessment of sustainable development of society. It is therefore integrated more
and more in decision making, where especially the Carbon Footprint is the exponent of the increasing popularity of LCA. However, several methodological choices still cause discrepancies in the final results, where the allocation procedure is a key issue. Much can be learnt from the current situation, where different regions propose new Carbon Footprint methodologies, with their own specific methodological choices. This causes problems when incorporating LCA results, such as the greenhouse gas (GHG) savings of biorefineries. In this work, a case study is executed in a Belgian multi-output biorefinery producing a mix of food, feed and fuel. Results, calculated with a black box and subprocess approach for economical value, mass, energy and exergy allocation, show discrepancies of up to 59% between the same procedure in subprocess and black box allocation, with a factor 4.1 difference within black box allocation approaches and 1.8 within subprocess allocation. The GHG savings of the biofuel versus the fossil fuel equivalent range from 0-50%. When considering a broad waste definition, as is suggested by some methodologies, GHG savings range up to 80%. This work highlights that the subprocess approach is preferred, as this reflects reality better. Furthermore, physical relationships, and especially exergy, are very useful in allocation, as economical values fluctuate in time, and can cause difficulties in finding representative values of intermediate flows in biorefineries.

MO 099
A mini-Delphi approach to consensus
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The Delphi method was originally developed as a forecasting method in which a panel of experts is used in a structured manner aiming to a common understanding. We used a version of Delphi to resolve a methodological problem where there is no objectively correct solution: deciding the primary energy factor of industrial waste heat, heat generated as byproduct, and heat produced from waste, when these are used for district heating in Sweden. In this context, the aim was not to find an accurate answer but to take advantage of the structured Delphi procedure, including its psychological effects, to reach consensus. Our approach was a mini-Delphi: a one-day workshop with 12 participants who were experts in one or more different roles of stakeholders. After an introduction to the methodological approach and types and to the mini-Delphi approach, the participants discussed the primary energy for the heat source in three groups to improve their understanding of the issue. Each participant then individually put a sticker on a numerical scale to represent the primary energy factor that the participants felt appropriate for the heat source discussed. When all participants did not immediately agree, the participants with the extreme values where asked to justify their decision. All participants where then allowed to revise their decision. Only one such iteration was made for each heat source due to the time constraints of the workshop. The approach yielded consensus on that waste heat should carry zero, and that heat as a byproduct should carry only primary energy corresponding to the increase in primary energy demand of the industry selling the heat. The workshop also converged on the view that waste that should not be recycled should carry zero or very little primary energy when used as fuel. Similar exercises can be performed to search for consensus in other methodological issues such as allocation of emissions from multifunction processes.

MO 100
The Cereal Unit allocation as a new allocation procedure for agricultural life cycle assessments
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For agricultural life cycle assessments (LCA) several different allocation approaches are currently used. This leads to a broad range of results. In addition, parts of the environment are often not accounted for in agricultural processes due to their different scales. In this study, the Cereal Unit approach was used in bakeries and wheat straw used in biofuel production. The users of the individual co-products (baker and biofuel producer) typically do not know of each other and as a result the allocation methods for their individual LCAs are not aligned to each other. This can lead to a situation that the total allocation burden is inadequately distributed. Material and method: Specific requirements for a suitable allocation method for the agricultural sector are defined, which were used to identify the Cereal Unit (CU) as a promising parameter for an agricultural allocation procedure. Results and discussion: Currently available allocation methods do not satisfy all requirements for the wide range of agricultural products. Therefore, a new allocation approach based on the Cereal Unit is suggested. The Cereal Unit has been developed since decades for purposes of agricultural statistics and is optimized continuously. It is based on physical, chemical and nutritional properties. The Cereal Unit as a common parameter can be allocated based on one common parameter. Exemplary results will be shown, among others, for wheat, rapeseed and sugar beet. Conclusions and recommendations: The Cereal Unit allocation approach allows using one common allocation procedure within agricultural LCAs by meeting the requirements of this sector. This approach could help to solve agricultural allocation problems and might lead to more robust LCA results for services and products originated from agricultural raw materials. We recommend investigating the applicability of the Cereal Unit allocation approach for agricultural LCAs.

MO 101
Life cycle analysis (LCA) applied to process, integration of process modelling in the building of life cycle inventory - example of a bio-polymer production process
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Life Cycle Assessment (LCA) methodology is a well-established analytical method to quantify environmental impacts, which has been mainly applied to products. However, it also has the potential as an analysis and design tool for processes [1]. One of the interesting identified challenges of LCA applied to processes is the consideration of the operating conditions in the building of Life Cycle Inventory. It allows taking into account the process as complex systems instead of black boxes and considering the environmental impact of the used operating conditions. This could be then interesting in multi-objective optimization (e.g. technical, economic and environmental) of processes and help the stakeholder in decision making process at the time of scale up. This work is based on the case study of an existing pilot process for the production of a biopolymer from wheat straw and bran. An original production process, based on twin-screw extrusion and ultrafiltration processes has been developed. A first cradle-to-gate LCA has been lead in order to help the choise into different options for unit processes and then to identify the hotspots of the process. Then these have been modeled using Excel flowsheet, and twin-screw extrusion was studied through experimental design. Both of these tools provide inventory data for several operating conditions and have been coupled with LCA with the aim to obtained results about the environmental impacts of each process unit depending on its operating conditions. This work provided a new perspectives in the fields of LCA applied to process industry: instead of using the methodology as an assessment method coming at the end of the process design, these one are rather integrated at the early stage of process conception with other parameters like economics and technic.

MO 102
Exergy analysis and LCA - a design for environment approach of energy conversion processes
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LCA has been widely applied to design for environment (DfE) of energy conversion processes. It covers the environmental impacts from fuel consumption and the construction, operation, maintenance and disposal of plant components. However, due to the methodological scope of LCA, the environmental impact is related to the product of the energy conversion process, for example to the amount of electric energy generated by a power plant. Especially the functional interdependencies between the process components are not known due to the lack of a harmonized physical base of all modelled plant components. This gap can be closed by an exergy analysis which enables the allocation of environmental impacts to exergy streams as thermodynamical basis for the overall process. To minimize the environmental impacts all energy conversion processes have to be optimized with regard to their exergy streams and exergy destruction. A new methodological approach has been developed based on the combination of exergy analysis and LCA. The so called exergoenvironmental analysis assigns environmental impacts to exergy streams. It identifies the environmentally most relevant process components as well as possibilities for the optimization of plant components. As a case study, an energy conversion process consisting of a high temperature solid oxide fuel cell (SOCF) integrated with an algaltherm biomass gasification has been analyzed. The investigation reveals the components which have the greatest environmental impacts caused by theirthermodynamic inefficiencies and their life cycle (component-related) respectively. It is shown the distinctions between the results of the LCA and the exergoenvironmental analysis, as well as the influence of the exergy destruction on its environmental impacts.

MO 103
Hybrid approaches in life cycle assessment
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With the ultimate goal to assess the greenhouse gas emissions of the livestock supply chain in Italy, different life-cycle based methodologies were reviewed with the aim to apply the most appropriate one. Input-Output Assessment (IOA) and Life Cycle Assessment (LCA) are two of them, which have both been applied for this specific aim. Both these methodologies have limitations, and when used alone, have some shortcomings. Hybrid approaches have the advantage of incorporating the details of a product perspective given by LCA, with the completeness of the economy-wide accounting of IOA. Therefore, theoretically they are considered a promising approach for linking the micro (product) with the macro (economy-wide) level, which is the core of our goal. Several Hybrid methodologies have been proposed, with not always a clear distinction among them. We identified three main categories, namely Tiered Hybrid Analysis (THA) proposed among others by Tieloar et al. (2004), Input-Output Hybrid Analysis (IOHA) reviewed by Joshi (2000) and Integrated Hybrid LCA (IHLC) developed by Suh and Huppes (2005). IOHA is the most applied followed by THA and IHLC, in the area of energy, forest, fuel and waste. Hybrid methodologies have been used for the comparison of different life cycles as organic and conventional farming techniques, and for the environmental assessment of regions and countries. When compared, Hybrid methods often show a higher emission level then LCA or IOA, explaining why they are often considered as a more complete assessment. However, this higher value can also be caused by double counting...
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MO 106 Material flow management of biogenic municipal waste: assessment of different technology options by means of LCA - to identify the appropriate reference system, and incorporated. The life cycle inventory can be further translated into the associated impact using a damage assessment model.

The results are part of the project BioEnergieDat, whose goal is to provide a database with harmonized, validated LCI data concerning provision of bioenergy under German European waste legislation. Currently, the German law on the collection and utilization of waste (Kreislaufwirtschafts- und Abfallgesetz (KrW-/AbfG)) is in the process of amendment to comply with the international environmental policy strategies pursue efficiency, to minimize monetary cost, to achieve social acceptance, among others. At the same time, the degrees of freedom and type of decisions to be taken in waste management are determined by regulations. The optimization of the environmental impact of waste management considering all the aforementioned limitations and constraints imposed by the regional context is hence a challenging task that requires advanced decision-making tools.

To tackle this challenge, a systematic tool based on a rigorous mathematical programming approach was developed. The optimization problem was posed in mathematical terms as a linear programming (LP) model, comprising two main sets of equations. The first includes mass and energy flow-based process models of common treatment options, including both dedicated waste treatments and co-processing activities. These models enable the quantification of the direct emissions and the resources consumed in terms of a target function that contains the life cycle inventory (LCI) of inputs and outputs associated with the system operation. The life cycle inventory can be further translated into the associated impact using a damage assessment model.

The model output is the optimal allocation of waste and resources to the available or planned waste treatment infrastructure. This allocation should ensure a given required functionality of the resulting waste treatment and industrial productivity while conforming to the constraints imposed by the regional context. The capabilities of the approach presented are illustrated through a case study. Numerical results highlight the importance of considering multiple objectives to avoid shifting burdens between impact categories. In addition, the environmental relevance of shadow prices, i.e., the marginal environmental impact of tight constraints, is discussed as an approach to analyze sensitivities and identify opportunities for improvements. The ultimate purpose of our tool is to guide policy makers and practitioners towards the adoption of waste management patterns with improved environmental performance.

MO 107 Material flow model of biogenic municipal waste: assessment of different technology options by means of LCA. W.B. Roland

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Generally, biogenic municipal waste can be burned, composted or fermented. Currently, in administrative districts that do not collect biogenic municipal waste separately, the generated electricity and heat are supplied to public. In administrative districts with a separate collecting system the biogenic municipal waste is composted. Composting plants are sold as fertilizer or soil enrichment to horticultural, agricultural and gardening companies, retail sector or private consumers. Since most of the plants are 10 to 15 years old, they need to be upgraded. A technical possibility of upgrading includes the generation of biogas from waste. The digested fuel can be either used to generate compost or be used to generate electricity and heat. Generating compost could result in an increased potential to convert energy from waste.

The objective of the poster presentation is to present, assess and compare from an environmental point of view the current state of the art of technologies to ferment biogenic municipal waste and to use the residue as compost for German requirements. The system boundary of the assessment includes the collection of waste, the fermentation and emissions is 0.35 for housing and 0.66 for mobility. This suggests that environmental impacts from housing are generated more equally than environmental impacts from mobility. The model is a promising approach to characterize the environmental performance of communities based on their inhabitants, to show graphically where highly emitting households are located, and to support local decision-makers by indicating households with big leverage potential for the reduction of environmental impacts in their community.

MO 109 Carbon flows from land use changes in LCA on bioenergy as a function of biomass demand and spatial allocation of land. V. Prokopy, J. Schielen, D. Sartorius, D. Schäfer, M. Kratz, P. Schiebel

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Due to the increasing share of solar and wind power generation, the load management needs flexible options to manage fluctuations in power supply as well as in power demands. To handle these fluctuations, a new kind of power plant is in development at Karlsruhe Institute of Technology. This power plant consists of a moving grate which is fueled by biogenic fuels with a high nitrogen content. The nitrogen is emitted as ammonia, which is captured and converted to liquid ammonium. The black ashes are used as fertilizer and the ash is sold as fertilizer or soil enrichment to horticultural, agricultural and gardening companies, retail sector or private consumers. Since most of the plants are 10 to 15 years old, they need to be upgraded. A technical possibility of upgrading includes the generation of biogas from waste. The digested fuel can be either used to generate compost or be used to generate electricity and heat. Generating compost could result in an increased potential to convert energy from waste.

The objective of the poster presentation is to present, assess and compare from an environmental point of view the current state of the art of technologies to ferment biogenic municipal waste and to use the residue as compost for German requirements. The system boundary of the assessment includes the collection of waste, the fermentation and emissions is 0.35 for housing and 0.66 for mobility. This suggests that environmental impacts from housing are generated more equally than environmental impacts from mobility. The model is a promising approach to characterize the environmental performance of communities based on their inhabitants, to show graphically where highly emitting households are located, and to support local decision-makers by indicating households with big leverage potential for the reduction of environmental impacts in their community.
The use of Life Cycle Assessment (LCA) is mandatory. Yet, potential pitfalls and methodological limitations exist in the application of LCA to CCU: the CO2 capture effect is easily misevaluated; the CCU system is inherently multi-functional; and the important criterion of CO2 fixation duration is not considered in a static, attributional LCA. This reduces CO2 emissions and therefore, the global warming potential in comparison to the other concepts. Furthermore, the primary energy demand and the acidification mechanisms of the Solar2Fuel concept can reduce emissions if membranes are used to capture CO2. In contrast, the CCS concept can only reduce greenhouse gas emissions if the power grid is decarbonized.

Therefore, limitations and hypotheses should be established to make the LCA methodology usable and relevant in view of the objectives and the applicability of the expected CO2 fluxes.

Utilization of CO2 captured in coal power plants for photocatalytic methanol production - ecological implications

A. Trudewind, A. Schreiber, D. Haumann, G. Göttlicher

because LCA conclusions can strongly influence decision makers, it is critical that regional heterogeneity is captured in data used for LCA metrics and for simulating market mechanisms. Geographic Information Systems (GIS) provide an opportunity to capture regional variations. Our case study is the Lawrence Berkeley National Laboratory LCA of pressure management for geologic carbon dioxide capture and sequestration (CCS). The objective of this study is to evaluate the regional variability of economics, environmental impacts, and risks of a pressure management technique involving brine extraction. We select three saline aquifers targeted for CCS from different geographic regions in the USA to assess brine extraction and disposal scenarios. GIS databases are queried for data from those three regions to perform network analysis. Initial calculations were performed assuming ten GW-size coal fired power plants were injecting 90% of their CO2 emissions into a saline aquifer CCS site with an annual injection of nearly 90 million tonnes of CO2 and an annual brine extraction of nearly 200 million gallons. We find that impacts and the potential commercial value of brine vary substantially between regions due to differences in brine composition and in market data. Water residing in the three saline aquifers spanned a range of temperatures and compositions. Economic value was sensitive to regionally specific electricity providers, salt and mineral markets, and water scarcity. Net values for management scenarios ranged from a cost of $590/CO2 to a return of $900/CO2. The unexpected finding that pressure management could mitigate the cost of CCS in certain regions of the USA would not have been recognized if national averages were used in calculations. While average values may not be reliable to the order of magnitude required for scenario modeling. The quantification of economic mechanisms and regionally specific market data using GIS provides a more spatially resolved alternative to current national-scale input/output economic sectors in LCA.

Life cycle of a product is generally characterized by the main following stages: Raw materials acquisition, Manufacturing, processing and formulation, Distribution and transportation, Use, re-use, maintenance, Recycle and Waste management. Considering the life cycle thinking in a risk analysis approach requires the adjustment of the classic risk analysis methodology. In order to build up this new methodology called Life Cycle Risk Assessment (LCRA), we relied on the Life Cycle Assessment (LCA) methodology, which is the state-of-the-art method to calculate the potential environmental impacts and to estimate the LCA of a product. The LCRA new methodology is explained and applied to two energy pathways for transportation sector: hydrogen (produced from the biomass) and gasoline pathways. The life cycle thinking is not taken into account in the traditional method of risk analysis. To integrate this fundamental concept to the risk analysis methodology following the LCA methodology, we defined three sub-steps for the assessment step: classification, characterization and valuation, and allows a conversion of inventory data into results of impact / risk levels. For LCRA, the data collected are only qualitative since it is an inventory of change mitigation potential. Each of the LCRA's four steps has its counterpart in terms of LCRA (Life Cycle Risk Assessment). The comparisons between these two methodologies reside on two key steps of the LCA: the inventory (step 2) and the assessment (step 3). The inventory collects data whose nature is different between the two tools. For LCA, data collected are matter and energy flow; whereas, the LCRA considers step classification, characterization, and valuation, and allows a conversion of inventory data into results of impact/risk levels. For LCA, the conversion of inventory data is performed by a calculation using characterization factors. For LCRA, this conversion is done qualitatively by rating and prioritizing risks. The LCRA's sub-step classification step for the prevention focuses on both the inventory and the impacts/risk to be assessed. Just like the flow identified by a LCA that can contribute to different categories of impacts, dangerous situations can cause different types of accidents. Therefore, limitations and hypotheses should be made for the LCRA methodology usable and relevant in view of the objectives and the applicability of the expected results.

Adapting life cycle assessment for multi-criteria analysis of a complex system: case study of urban mobility

L. De Fournon, L. De Boulch, V. Laroche, N. Gondran

Urban mobility is identified as one of the most CO2 emitters in France: transport represents 34% of CO2 emissions in France in 2006 (ADEME 2006), including 25% of urban trip emissions. Some previous studies compare transport modes (Finkbeiner & Al 2006), or assess CO2 emissions that are related to urban mobility on a real case
INSEE (2011). But literature is lacking about multi-criteria analysis of urban mobility in the general case. In particular, Life Cycle Assessment has not been largely used to qualify urban mobility impacts despite its possibility to evaluate the environmental impact (ISO14040, 2006).

Nevertheless, the complexity of the system "urban mobility" is a first difficulty to the evaluation, especially concerning the definition of goal and scope. The main topic of this paper is to find a way to complete and adapt LCA in order to enable the evaluation of a complex system under the case study of urban mobility. We propose an approach based on the System Analysis Design Technique (SADT) that allows a clear and complete definition of the "urban mobility" system. Then the possibility to include more societal indicators beyond the environmental ones (such as noise, satisfaction of consumers, time travel, costs etc) will be studied. The final aim is to provide a configurable approach for different scenarios of urban mobility.

The first results consist of a complete definition of the system that is based on a segmentation of urban mobility into sub-systems that constitute the "first and objectives" step in LCA. This decomposition prepares to the next step of LCA, in which modul projects will be aggregated with elementary assessments of modes to obtain a multi-criteria analysis of several scenarios.


MO 115

LCA of land-based freight transportation: including accidents in LCA

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The analysis combined human health impacts generated with those assessed by the system approach. The aims were to compare the European trends for transport by road with other forms of vehicle efficiency and emissions, and this in the context of the uncertainty surrounding the determination of emissions-related impacts. From an LCA perspective, the use of road vehicles causes emissions from the up and downstream stages of the vehicle and vehicle fuel life-cycles as well as during use, and by applying conventional LCA methodologies it is possible to assess the impact on human health of this category of pollutants. For exposure especially in the form of human health effects, it is clear that the occurrence of road traffic accidents have a severe consequence for the occurrence of road traffic accidents has a severe consequence for the occurrence of road traffic accidents. The accident data on background processes was missing, the analysis could only be performed for the operation phase of the vehicles. Nevertheless, the relevance of accident data in traffic operations could clearly be demonstrated for road traffic indicating that accidents should be routinely considered in LCA studies in order not to miss impacts of high relevance.

Please see extended abstract for detailed information.

MO 116

Trends in transport-related human health impacts from life-cycle emissions and road accidents in Europe

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The analysis combined human health impacts generated with those assessed by the system approach. The aims were to compare the European trends for transport by road with other forms of vehicle efficiency and emissions, and this in the context of the uncertainty surrounding the determination of emissions-related impacts. From an LCA perspective, the use of road vehicles causes emissions from the up and downstream stages of the vehicle and vehicle fuel life-cycles as well as during use, and by applying conventional LCA methodologies it is possible to assess the impact on human health of this category of pollutants. For exposure especially in the form of human health effects, it is clear that the occurrence of road traffic accidents have a severe consequence for the occurrence of road traffic accidents. The accident data on background processes was missing, the analysis could only be performed for the operation phase of the vehicles. Nevertheless, the relevance of accident data in traffic operations could clearly be demonstrated for road traffic indicating that accidents should be routinely considered in LCA studies in order not to miss impacts of high relevance.

MO 117

LCA of age-related environmental impact for biogenic hydraulic fluids

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3Biogenic hydraulic fluids, based on synthetic esters, have an excellent environmental profile in the unused state, so that they are typically classified as 'non hazardous to water'. During storage at room temperature and tribological application, occurring chemical and toxicological changes take no account in the classification of lubricating oils until now. The ageing and oxidation stability gets increasing importance, since it determines the service life of lubricants in tribological systems in addition to the storage time. With an increased use of biogenic hydraulic fluids in environmentally sensitive areas, thus the need for an appropriate monitoring and assessment approach as part of a Life Cycle Assessment (LCA).

A lubricating oil mixture, based on high oleic sunflower oil, was monitored in an adapted life cycle for biogenic hydraulic fluids. For the detection of ecotoxicological effects, water soluble fractions (WSF) of oil samples with a real effect concentration of 100 g/L were prepared. Ecotoxicological testing was performed in standardised bioassays using 24- and 96-well microplates for algae and bacterial growth inhibition tests. Biodegradability was determined with the optimised test system "O2/CO2-Headspace Test". After tribological application in an ageing test bench (48-192 h) there was only an increasing toxicity for the algae growth inhibition test as a function of time in contrast to the unused fluid with EL50-values ranging from 80 % to 5 % (192 h). The ecotoxicity of hydraulic fluids increased depending on storage conditions. Storage at room temperature accelerates this process compared to 4°C. Compared to ecotoxicological behaviour by ageing during storage and use, a readily biodegradability within 28 days (≥ 70 %) was determined as it is demanded for the environmental label "Blue Angel" for biogenic hydraulic fluids. Due to the associated metal content with ICP-MS in used hydraulic fluids and resultant WSF as well as the determination of the aqueous available metal content, there is a better interpretation and distinction of age-related aquatic ecotoxicity. The additional characterisation of conditional-use changes in ecological characteristics allows a comprehensive assessment and is still largely unknown, but an important aspect in terms of LCA for biogenic hydraulic fluids. This methodological approach thus allows the feedback of the desired product properties on the production method.

MO 118

Hydrodried vegetable oil production in Spain: compliance with Directive 2009/28/EC in terms of GHG emissions savings

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Oil hydrodrying units in refineries are aimed at reducing the sulphur content of mineral fuels in order to get compliance with standard specifications. Moreover, this process is one of the best available technologies to obtain biodiesel from vegetable oil at present. This study presents the GHG emissions of the biofuel HVO (Hydrotreated Vegetable Oil) obtained from vegetable oil by means of co-processing with conventional fossil fuel in hydrodrying facilities of two crude-oil refineries, using the LCA methodology. Results were compared with other studies (in the same system boundary) and with the Life Cycle Inventory for the production of hydrodried vegetable oil. Results highlight that the HVO mixed with mineral diesel has at least the same environmental performance compared to a mixture of FAME and mineral diesel. GHG emissions savings compared to standard diesel from Renewable Energy European Directive 2009/28/EC are exposed. In addition, sensitivity analysis have been conducted in order to assess the influence in results of different data sources and different allocation processes that LCA standards suggest, showing the most relevant hot-spots that influence the improvement of previous results.

MO 119

Life cycle assessment of chitosan-based films

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Conventional plastic films used nowadays for food packaging are based on non-renewable sources, non-biodegradable and impact heavily on the environment. For all these reasons, a new biodegradable packaging material based on renewable raw materials has been developed. Chitosan is the second most abundant polysaccharide found in nature, has non-toxic, biodegradable, and antimicrobial characteristics, which are of great interest for food packaging purposes. Chitosan is mainly manufactured from crustaceans due to the large amount of its skeleton available as a by-product of food processing. In this context, chitosan is an interesting film forming material. A comparative life cycle assessment between two different packaging systems has been carried out: a commercial food packaging film based on polypropylene (PP) and a new biodegradable, chitosan-based film manufactured in our labs. The environmental load of chitosan-based films in different stages of its life cycle has been studied and compared with the one of conventional PP films used as food cover.

The functional unit considered in this study is 1 m2 of similar thickness packaging film. The studied system includes three main stages: material obtaining, film manufacture, and end of life. The data relating to PP packaging films were obtained from Ecoinvent v2.0 database, developed. The life cycle inventory for chitosan powder from crustacean shell was taken from literature and film manufacture data was measured directly by our research group in the lab. The Ecoladicator 99 method was selected for comparison of the films.

Obtained results showed that PP film has a higher impact than chitosan-based film in carcinogens and fossil fuels impact categories. The environmental burden associated to carcinogens is mainly due to the end of life stage, while the impact related to fossil fuels is owing to the extraction of PP. On the contrary, the categories in chitosan-based film has a higher environmental load are respiratory inorganics, land use and minerals. Environmental load associated to respiratory inorganics is mostly related to the
acetic acid used in film manufacture and, in a significant way, to the hydrochloric acid used in the raw materials extraction, which is also responsible for the impact in minerals category in the raw materials extraction stage. In addition, the main responsibility for the land use is given to the use of virgin pulp and transportation processes, where the greater environmental impacts were located.

MO 121

GHG emissions comparison of tissue paper from virgin pulp vs. recycled waste paper
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Pulp and paper manufacturing industry is the fourth largest greenhouse gas (GHG) emitter and is responsible for around 9% of the total global CO2 emissions from manufacturing sectors. The increasing trend of paper consumption is one of the principal drivers behind the emissions from the sector. Therefore, policy measures which ensure not only the reduction in consumption of paper but also the increased use of recycled waste paper, the implementation of cleaner production practices and the use of fibres from sustainable sources are of main importance.

The Life Cycle Assessment (LCA) is used for this study with the objective of making a comparison between the use of virgin and the use of recycled raw materials in the production of corrugated cardboard, under Mexican conditions. The contribution of applying LCA works to identify different environmental impacts from the individual processes of the complete system.

The identification of the environmental impacts runs in order to consider specific aspects, which would focus on the sustainability of the enterprises involved in the corrugated cardboard industry. The aspects identified could be properly implemented in the future as a functional part of an environmental management system.

The results showed that the system of the recycled corrugated cardboard production impacts less in every environmental impact category analyzed, than the virgin corrugated cardboard production. However, in Mexico, it is a system that can be optimized through specific activities; especially in the manufacturing and transportation processes, where the greater environmental impacts were located.

MO 122

Combination of Planar Chromatography with in vitro bioassays as a tool for effect directed analysis
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The Life Cycle Assessment (LCA) is used for this study with the objective of making a comparison between the use of virgin, and the use of recycled raw materials in the industries in the country is essential to achieve tangible results for the environmental welfare.

Considering the present situation in Mexico, the environmental protection and the responsible use of the available resources are current needs. The involvement of each of the industries in the country is essential to achieve tangible results for the environmental welfare.

The Life Cycle Assessment (LCA) is used for this study with the objective of making a comparison between the use of virgin and the use of recycled raw materials in the production of corrugated cardboard, under Mexican conditions. The contribution of applying LCA works to identify different environmental impacts from the individual processes of the complete system.

The identification of the environmental impacts runs in order to consider specific aspects, which would focus on the sustainability of the enterprises involved in the corrugated cardboard industry. The aspects identified could be properly implemented in the future as a functional part of an environmental management system.

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MO 123

Development of a multi-component detection method for phthalate di- and mono-esters using simultaneous analysis technique and UPLC-tQ-MS/MS to identify steroid hormones in CALUX positive surface water samples
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Surface waters, such as the rivers Rhine and Meuse, are important sources for the drinking water supply in the Netherlands. Dutch drinking water companies therefore intensively investigate their water sources for the presence of emerging contaminants and their fate during treatment processes. One of the classes of concern are compounds with endocrine disrupting, e.g. estrogenic, activity. Nowadays, natural and synthetic steroidal estrogens are known as the primary causative agents of estrogenic activity in the aquatic environment. With the development of new bioassays, it has become possible to investigate also the aquatic activity of other classes of steroid hormones and compounds with comparable or antagonistic activities. Many hormone-like compounds are excreted naturally or are used as pharmaceuticals and might thus enter the environment via similar routes as estrogens. Indeed, using CALUX bioassays for progestagenic, androgeneic, estrogenic and glucocorticoid receptor activation, the presence of especially glucocorticoid activity in Dutch surface waters and glucocorticoids in waste water was recently reported. Now that activity is found, methods are needed for the identification of the responsible compounds.

In order to overcome these difficulties, environmental samples are usually concentrated by solid phase extractions (SPE). However, potential active compounds which are not bound to the matrix of the solid phase are not concentrated and so the SPE fraction might already result in a separation of the sample. They might be even lost if the flow-through of the SPE is not further characterized.

As a consequence, we have developed a method described above is the use of planar chromatography (HTLC) in combination with bioassays. Samples can be easily concentrated on a thin layer plate by multiple sample application and/or a focusing step if a multi development method is used for the chromatography. The challenge of this strategy is to perform bioassays for the detection of specific effects directly on the surface of the thin layer plate. Therefore, these bioassays have to be adapted for this new application.

The combination of thin layer chromatography with the luminous bacteria test for the detection of acute toxic effects is already well described and frequently used. In contrast, studies showing the coupling of HTLC with specific bioassays like the Yeast Estrogen Screen (YES) are scarce. The objective of the presented work is to develop an optimized technique for the combination of HTLC with various specific bioassays and to test the hypothesis that such an approach is a useful tool for the directed analysis of environmental samples.

MO 124

Development of a multi-component detection method for with UPLC-tQ-MS/MS to identify steroid hormones in CALUX positive surface water samples
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Simultaneous analysis technique for trace amount of phthalate di- and mono-esters was developed and applied to aqueous samples. Phthalate esters are used as plasticizers and often reported to have endocrine disrupting activities. When phthalate di-esters are taken up into the body, they are metabolized into mono-esters and excreted to urine. Since both di- and mono-phthalates are likely to exist as mixture in the environmental water and wastewater, it is important to develop a simultaneous quantification method for both forms. Researchers reported the advantage of using on-line SPE system coupled with LC-MS/MS determination which gives easy and high throughput analysis. This technique also provides greater reproducibility of pretreatment processes. To achieve high throughput analytical methods, not only the on-line SPE system but also high speed mass spectrometer is required. Phthalate esters have various homologues with different alkyl side chains. Positive ionisation is more suitable for phthalate di-esters while negative ionization for mono-esters. The mass spectrometer used in this study were capable of high speed MRM (500 ch/s) and polarity switching (15 ms). This presentation reports simultaneous analysis of the phthalate di- and mono-esters using online-SPE system and simultaneous positive/negative electro-spray ionization.

MO 125

Synthesis and identification of novel metabolites of polychlorinated diphenyl ethers in human blood
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1Stockholm University, Stockholm, Sweden
2Department of Chemistry, University of Chemical Sciences, Uppsala, Sweden

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MO 126

Isolation and identification of ligands for the goldfish testicular androgen receptor in chemical recovery condensates from a Canadian bleached kraft pulp and paper mill


Institute for Environmental Studies, Amsterdam, The Netherlands

Biodetection System, Amsterdam, Nederland

This study builds on a series of investigations characterizing substances in milk and other food samples that may have estrogenic activity. The authors have identified androgenic compounds in the chemical recovery condensates from a Canadian bleached kraft pulp and paper mill. These compounds were identified using high-resolution mass spectrometry and compound lists and mass spectrometric libraries were used to identify the unknown compounds. The study highlights the need for further investigation to identify the potential risks of these compounds.

MO 127

Thyroid hormone disruption in effect-directed analysis - An endpoint of growing concern


Institute for Environmental Studies, Amsterdam, The Netherlands

Biodetection System, Amsterdam, Nederland

Thyroid hormones (THs) play an important role in the maintenance of a normal physiological state. Chemicals in the environment that interfere with the ability of the thyroid hormone system to function normally could have devastating effects on individuals, as well as on whole populations. THs are important in regulating total body metabolism as well as the transport of the THs, which includes binding to the TTR (thyroxin transport receptor). The presence of functional groups together with the length of the fluorinated carbon chain was a crucial factor for binding to the TTR binding site. The presence of functional groups together with the length of the fluorinated carbon chain was a crucial factor for binding to the TTR binding site. The presence of functional groups together with the length of the fluorinated carbon chain was a crucial factor for binding to the TTR binding site.

MO 128

Estrogenicity of 75 European waste water effluents evaluated by in vitro assay

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Various natural and synthetic contaminants present in urban waste waters have been shown to posses estrogenic activity, and many questions related to the risks of these compounds remain to be addressed. Under environmental conditions, steroid hormones appear to be primarily responsible for adverse effects observed in fish downstream of waste water treatment plants (WWTPs). However, instrumental analyses of steroid estrogens often fail in detection of their actual concentrations in complex environmental matrices such as waste water effluents. In the present study, MLV in vitro assays were used to evaluate estrogenicity of 75 European waste water effluents. The samples originated from SEES 2010 pan European monitoring campaign coordinated by the European Commission\'s Joint Research Centre (JRC) in Ispra, Italy. Estrogenic activity was expressed as 17β-estradiol equivalents (EEQ). Twenty seven sample extracts showed significant estrogenic activity higher than the detection limit (0.5 ng/L EEQ) with values ranging from 0.53 to 17.9 ng/L EEQ. The highest activities have been detected in WWTPs at some of the major European capital cities indicating the importance of this contamination source. This study provides some of the first EU-wide snapshot data on the estrogenicity of WWTP effluents, and demonstrates suitability of in vitro bioassays as effect-based monitoring tools.

MO 129

Assessing the endocrine activity of sediment samples from Laguna Lake, Philippines, using the LYES and H295R assays


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UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany

University of the Philippines, Manila, Philippines

University of Leipzig, Leipzig, Germany

Due to the increasing use of different chemicals by modern society, nearly every living space is affected by numerous known and unknown chemical contaminants. Since the early 1990s there is increasing concern about the exposure to chemicals that have the potential to interact with the endocrine system and associated physiological functions. The present study was conducted to further investigate the estrogenic activity of sediment samples from Laguna Lake. The lake is the second largest freshwater lake in Southeast Asia and the largest lake in the Philippines. It is also a member of the Living Lakes Network since 2001. Based on preliminary results, the present study assessed the potential of sediment samples to disrupt steroidogenesis pathways. The sediment samples showed estrogenic activity, and further investigation is needed to determine the potential risks of these compounds.

MO 130

Effect of 4-nonylphenol and/or diisononylphthalate on TH-17 cells: effects of estrogenic disruptors on human immune system parameters

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Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic

The present work was to investigate the impacts of two estrogenic disruptors compounds (EDCs) and the human immune response through a study of their effect on the TH-17 human cell line which was used as a model for macrophages. We used two EDCs, diisononylphthalate (DINP) and 4-nonylphenol (NP) alone or in combination to evaluate the effects of these compounds on several parameters of the immune system: cytokine secretion, phagocytosis, and the putative implication of the estrogen receptors by studying the level of MAPK activation. NP and DINP strongly reduced phagocytosis and modify cytokine secretions. Indeed, TH-1 cell suppressions (i) and 10 μM of combination of NP and DINP induced an IL-8 level in the medium respectively 28.9 and 54% higher than level obtain for control (untreated cells), (ii) to combination...
Rapid determination of bisphenol A in water by amperometry using a tyrosinase biosensor

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Bisphenol A (BPA) has been considered significant because of its interfering the endocrine system of wildlife and human, increasing cancer rate, etc. In this work, we prepared a novel electrochemical biosensor for the rapid determination of BPA.

A 10μL aliquot of 1.0mg/mL the treated multiwalled carbon nanotubes (MWNTs) solution was cast on the surface of a glassy carbon electrode (GCE), dried in air. Gold nanoparticles were electrodeposited onto the MWNTs modified electrode by amperometry at 1.5V for 5min from a fresh colloidal gold solution which was prepared with 0.01% HAuCl4. After being washed with deionized water, the electrode was washed with ethanol as Au/MWNTs/GCE. A 7μL aliquot enzyme solution containing tyrosinase (Tyr) and Silk fibroin was then dropped on the surface of the Au/MWNTs/GCE, allowed to dryness at room temperature. This enzyme activity was approximately equal to 40U on each electrode surface. All surface electrochemical measurements were performed with CHI650c electrochemical analyzer. A bare or film modified GCE with a diameter of 3 mm was used as working electrode, a platinum electrode and a saturated calomel electrode was used as counter and reference electrode respectively.

The SEM image of the Au/MWNTs/GCE showed that the diameter of the Au nanoparticles dispersed on the MWNTs was about 10 nm. In the presence of molecular oxygen, the tyrosinase catalyze the oxidation of BPA to quinones. The experimental results of cyclic voltammetry showed that the reduction peak current increased with the BPA concentration. The MWNTs and gold nanoparticles possessed excellent synergetic electrocatalytic effect to enhance the electron transfer rate. The amperometric cardiac current is proportional to BPA concentration in 0.1M pH 7.5 phosphate buffer solution over the range from 0.2μM to 8.0μM with a correlation coefficient of 0.9991 and a detection limit of 0.1μM. The method was applied to determination of BPA in real water samples. The experimental results showed the biosensor possessed high sensitivity, good selectivity and stability towards the determination of BPA.

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MO 133 Evaluating endocrine activity in plant leaf extracts: temperature and solvent choice influence androgenic, oestrogenic and progestogenic activity in vitro and in vivo

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The goal of this research is to characterize the in vitro and in vivo activities of plant-derived endocrine active compounds (EACs) and to evaluate potential effects of representative plant extracts on fish and mammalian reproductive and neurobehavioral health. In contrast to mammalian systems, plant-derived EACs are an underexplored source of potential endocrine disrupters in fish species and their effects require further investigation. Scientifically reliable assessment of the potential impacts of synthetic EACs on fish health therefore depends on a holistic assessment of cumulative aquatic exposures to EACs of specific modes of action pertinent to fish reproduction (especially androgens, oestrogens and progestins). Currently, leaf material was collected from three woodland and marsh sites in south west England and processed for screening using a battery of (anti)estrogenic, (anti)androgenic and (anti)progestogenic in vitro screens (the YES, YAS and YPS assays, respectively). Key factors that have been addressed during the first phase of work are the impact of temperature and solvent on the extraction of dissolved organic carbon (DOC) and consequent responses in the YES, YAS and YPS assays (as both agonism and antagonism). For each plant sample, heated, ocean (Fagus sylvatica), oak (Quercus robur), birch (Betula pendula) and willow (Salix caprea) were exposed to 24°C and 4°C of direct sunlight for 12 or 24h after homogenization in water for 24 h at 20, 15 or 4°C. The aqueous extracts (approx 2L) were stored at -80°C and subsamples used to measure DOC. Aqueous extracts (10 μL volumes) were directly screened in the YES, YAS and YPS assays. In addition, aqueous extract subsamples (10 μL) were concentrated by solid phase extraction (SPE) onto Sep-Pak® Plus cartridges. The cartridges were eluted with 1 μL methanol and the extracts then also screened in the YES, YAS and YPS assays. Extraction temperature had a marked proportionate effect on aqueous DOC values. For the reed aqueous extracts, there was a DOC-related response in the YES assay. For the oak and eche extracts, there were variable responses in the various yeast assays. The information generated in this study will be used to support further characterisation of EACs in plant leaf material using a range of in vitro screens and in vivo fish test protocols. This work is funded by the CEFIC Long-range Research Initiative (RP reference EMSG55).

MO 134 Development of an in vitro model for screening EDCs and deciphering the specific target sites along the HPG axis

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Currently, the vast majority of studies on endocrine disrupting chemicals are based on in vitro approaches. The intrinsic complexity of the neuroendocrine and endocrine systems in in vivo systems often makes it difficult to understand the total effects of endocrine disrupting chemicals (EDCs) in specific organs. As such, studies using in vitro systems are required to provide information complementary to in vivo studies, and to provide important insights into the action mechanisms of EDCs in specific organs. We have successfully developed primary cell cultures (pituitary, ovarian follicular and testicular cells) in the marine medaka (Oryzias melastigma), a unique non-mammalian model system. This approach has allowed us to develop a series of experiments that evaluate the effects of different EDCs at different levels of the medaka HPG axis, particularly on steroidogenesis in the gonads. To validate these systems, individual cell types were challenged with environmentally relevant concentrations of common EDCs including polybrominated diphenyl ethers (PBDE), 4-nonylphenol (NP) as well as hexafluorophosphate. The expression levels of various steroidogenic genes (including cyp19a, cyp11a, 20bhsd, cyp19b, cox1, cox2, etc.) were analyzed by quantitative real-time RT-PCR. Among the 12 genes genes studied, the aromatase (cyp19a) was responsive to almost all treatments, suggesting that it is a major target site for endocrine disruption. The results obtained from these in vitro studies were compared to those obtained from in vivo exposure of whole fish as well as from H293R, a human cell line. Although different systems did not generate identical expression responses to the same chemical exposure on all parameters, the results suggested that medaka primary cell cultures not only serve as a useful tool for screening EDCs, but also provide a platform for deciphering the specific target sites of EDCs along the HPG axis and their action mechanisms.

MO 135 TTR binding of metabolite extracts from biological matrices: identification, quantification and TTR binding potency of co-extractants from microsomal extracts

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Free fatty acids interact with transport proteins like transthyretin (TTR), and with the hormone thyroxin (T4) interfering with the competitive binding and ultimately preventing the analysis. However, it is not exactly known which are the co-extracted compounds from various biological matrices that present a problem for the competitive binding analysis. We aimed to identify and quantify metabolite compounds from a biological matrix to their binding analysis to understand the problem dimension and its evolution. Incubations of rat liver S9 fraction were extracted according to published methods and co-extractants were identified and quantified through GC/MS analysis after fractionation. Saturated fatty acids (SFA), non-saturated fatty acids (NSFA) and cholesterol were identified as the prevalent co-extractants. At current dilution factors, co-extractant concentrations ranged from 8-26 μM of SFA, 2.5-5 μM NSFA and 0.2-1.4 μM of cholesterol. The potency of identified co-extractants was determined in a nA- well plate using the competitive displacement of the ANSA fluorophore (Ka = 1.5 μM) from TTR. Although less sensitive (T4 EC50 = 300 nM), this method is high
throughput and it does not require radioactive ligand or expensive equipment. SFAs partially displaced the fluorophore from TTR with an average EC50 of 1.0 μM. Given the typical co-extractant concentrations, the fatty acids in the extract are the most likely cause of the interference. Due to their physicochemical similarities, complete separation of POP metabolites from fat has been only possible through derivatization and acid clean-up, which will render the metabolites unsuitable for biological testing. As further dilution will weaken the detection limit, a method to selectively remove the fatty acids is currently under validation.

MO 136

Tetrabromobisphenol-A disrupts thyroid hormone receptor alpha function in vitro: use of fluorescence polarization to assay coactivator and corepressor binding

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Thyroid hormone receptors (TRs) recruit corepressor or coactivator factors to the promoters of target genes to regulate their transcription. Corepressors such as nuclear hormone receptor corepressor (NCOR) are recruited by unliganded TRs, whereas coactivators such as steroid receptor coactivator-1 (SRC-1) are recruited when trivalent TR ligands bind to the side chains of these receptors. These interactions depend on the ligand binding domain (LBD). The TRs via the use of consensus peptide sequences that can be used to probe the conformational changes induced in TR LBD by TR ligands. Recombinant LBD of the human TR 1 isoform (1TR 1 LBD) was produced as a fusion with glutathione S-transferase, and used to develop assays based on fluorescence polarization to quantify the binding of either NCOR or SRC-1 to TR. This condition condition obtained a reciprocal dose-dependent increase in SRC-2 peptide binding, in the woman cases at 50% effective doses. The TR agonists triiodothyroacetic acid and thyroxine were also effective in preventing NCOR peptide binding and increasing SRC2 peptide binding, whereas reverse-triiodothyronine was less efficient and the biologically inactive thyrione had no effect on either process. These experiments were based on the putative data that led to the proposal of the potential of TBBPA to bind to the TRs and modulate the expression of TR-regulated genes. However, further studies are necessary to better understand the mechanism of action of TBBPA in the TR system.

MO 137

The endocrine disrupting effect of hypoxia on pituitary cells

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Gammarus pulex has been used in the fully automated real-time based Multispecies Freshwater Biomonitor Trademark (MFB) to detect rapidly low concentrations of Bti fermentation products, one of which has been proven to be active in the LYES assay, for additional endocrine potentials including suppression of GnRH gene and promoter activity in pituitary cells. The present results indicate that TBBPA did indeed interfere with the ability of the 1TR 1 LBD to bind both NCOR and SRC-2. TBBPA behaved similarly to T3 in promoting the release of NCOR from LBD, whereas it failed to promote LBD interactions with SRC-2. However, it did reduce the T3-induced interactions between LBD and the coactivator peptide. This study therefore suggests that TBBPA in the micromolar range can affect the regulation of transcription by both the apo- and the holo-TR 1, with potential disruption of the expression of genes that are either up- or down-regulated by T3.

MO 138

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MO 139

Gammarus pulex responses to short-term exposure towards endocrine disrupting pharmaceuticals and chemicals in water

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Gammarus pulex is one of the most frequent and abundant freshwater amphipod in European streams, playing an important key-role in the aquatic food web and detritus cycling. Moreover, Gammarus pulex is sensitive towards pollution. Endocrine disruptors and pharmaceuticals are important chemicals in the effluent of urban waste water treatment plants, as well as a source of micropollutants which are not degraded during the biological treatment, e.g. filtration and solid phase extraction (SPE). EFSA and/or other agencies did not induce TR, RAR, or VDR activity. Compared to that, 54 of 78 effluents (69%) significantly activated RAR. This study indicates that TBBPA in the micromolar range can affect the regulation of transcription by both the apo- and the holo-TR 1, with potential disruption of the expression of genes that are either up- or down-regulated by T3.

MO 140

Do Bti fermentation products disrupt the endocrine system?

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Mosquitoes are well known as vectors for many pathogens, such as malaria (Plasmodium falciparum). Global warming and an increasing formation of potential breeding habitats are also a threat to the population of mosquitoes. Therefore, potential agents for the control of mosquitoes are indispensable. However, use of synthetic insecticides - such as DDT - bears the risk of causing serious damage to wildlife. As a consequence, there is need for alternative pesticides that effectively control pests without impacting the environment. Over the past three decades a protein from Bacillus thuringiensis israelensis (Bti) has been successfully used as a biological control agent against mosquitoes. However, the interference of mosquito, T3 induced a dose-dependent increase in the expression of GnRH mRNA, which is necessary for the induction and maintenance of GnRH gene expression. This study indicates that TBBPA in the micromolar range can affect the regulation of transcription by both the apo- and the holo-TR 1, with potential disruption of the expression of genes that are either up- or down-regulated by T3.
verify the results from the LYES assay using a breast cancer cell line (MVILN). Both products showed an increase of human estrogen receptor (hER) binding at the highest concentration during a pilot experiment.

**MO 141**

*Intersex in Scrobicularia plana: transcriptomic analysis reveals new genes involved in endocrine disruption*  
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MO 142

*Multigenerational and individual effects of endocrine disruptors on growth and reproductive endpoints using the copepod, Eurytemora affinis*  
T.L. Lesueur1, A.S. Sousis1, C.B. Boulanger-Lecomte1, S.S. Sousis1, Y. Pan2, E.P. Petrilli2, J. Forget-Lelat3  
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**MO 143**

*Photosynthetic and proteomic responses of the marine diatom Thalassiosira pseudonana to triphenylamine exposure*  
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**MO 144**

*Mechanisms of the neural disruptor group ‘selective serotonin reuptake inhibitors’ in Daphnia magna*  
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**MO 145**

*Effects of chronic exposure to gemfibrozil on aquatic organisms Pseudokirchneriella subcapitata, Daphnia magna, Moina macrocopa, and Oryzias latipes*  
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**MO 147**

*Endocrine disruption of chronic exposure to menefin acid on aquatic organisms Daphnia magna, Moina macrocopa, and Danio rerio*  
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Pharmaceuticals are trace contaminants of growing importance in aquatic ecosystem due to their physiologically active nature and their potential for impacts on non-target species. Menefin acid (MFA, CAS No. 50-78-2) is a non-steroidal anti-inflammatory drug (NSAIDs), which has been widely used for analgesic, anti-pyretic and anti-inflammation. Earlier, the authors evaluated the acute toxicity of menefin acid employing several aquatic organisms, including two cladocerans, Daphnia magna and Moina macrocopa, and a teleost, Danio rerio. The 48 h acute median effective concentration (EC50) of D. magna was determined at 17.16 mg/L. In 21 d chronic toxicity test, D. magna showed significant changes in reproduction related characteristics after the exposure. In the early life stage test using D. rerio, there was a significant decrease of larval survival at the lowest level of menefin acid. In addition, vitellogenin gene expression and Vtg protein level were reduced at the highest experimental concentration of menefin acid, 1 mg/L. The result of this study indicates that this pharmaceutical may have a potential effect on survival, reproduction and growth of the aquatic
The effects of nonylphenol ethoxylates having different ethylene oxide (EO) chain on fish, daphnia, and alga

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3Nonylphenol ethoxylates (NPEs) have been used as surfactants in detergent formulations in both of industrial and domestic. NPEs are biodegraded to shorter ethylene oxide (EO) or nonylphenol (NP). NP shows high toxicity to aquatic organisms, and also, NPEs having shorter ethylene oxide (EO) chain have been known to show greater toxicity than that having longer EO chain. It is important to examine the effects of not only NP but NPEs on aquatic organisms. However, there were little toxicity data on aquatic organisms of different EO chains. To examine the different effects of NPEs having various EO chains (NP1EO, NP2EO, NP3EO, NP4EO, NP5EO, and NP6EO) on aquatic organisms, we performed the acute tests using alga (P. subcapitata), daphnia (D. magna), and fish (O. latipes). Each acute toxicity test was carried out according to OECD guidelines. NP concentrations in test water were 50, 100, 200, and 300 mg/L. Changes of VTG level detected in female fish exposed to flutamide (nonsteroid anticancer drug) suggest U shaped concentration-effect curve. Decrease of GSI in male fish was also observed. VTG levels were increased in female and decreased in male fish corresponding with concentration of propiconazole (biocide, fungicide) whereas GSI in male fish was increased.

The study was funded by the Swiss National Science Foundation (project No. 31003A-121829 to K.E.)

MO 150
Effects of the UV filter benzophenone-3 in adult zebrafish (Danio rerio) and eleuthero-embryos at environmental relevant concentrations

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UV filters are widely used in consumer products. The photosensitizing compound 2-hydroxy-4-methylbenzophenone-3 (benzophenone-3, BP-3) is added to sunscreens and other personal care products to protect against UV damage. However, environmental and health effects of BP-3 have not been studied in detail. In order to test whether EDC-related effects are reversible, an exposure scenario limited to 60 d was followed by (a) a recovery period of 40 d or (b) continued exposure for 14 days and 120 hours post fertilization, respectively. Exposure concentrations of BP-3 are 10, 200 and 600 µg/L, as confirmed by LC/UV. The water analysis indicates that within 48 hours adult zebrafish, but not embryos, transform BP-3 in part to benzophenone-1 (BP-1), an estrogenic compound also used as UV filter. BP-3 and BP-1 are accumulated in fish up to 21 µg/g and 266 ng/g, b.w. respectively. We analyse molecular effect by whole-genome transcriptomics (microarrays) and find no significant transcriptomic changes after 96 hours post fertilization at 312 µg/L BP-3. 123 transcripts are altered belonging to different pathways. By applying a target gene expression approach focusing on the endocrine system in the brain, liver and testis of adult zebrafish and in embryos, expression of estrogen receptor, androgen receptor, and vitellogenin are determined by qRT-PCR. This indicates, in conclusion, the transcriptional response of zebrafish reveals key mechanism of genotoxicity. However, in this study, we show that both BP-3 and BP-1 have estrogenic effects in fish.

The results of this study suggest that both BP-3 and BP-1 have estrogenic effects in fish in vitro and in vivo. In our study, effects of BP-3 are evaluated after an exposure of adult male zebrafish (Danio rerio) and zebrafish embryos for 96 hours post fertilization, respectively. Exposure concentrations of BP-3 are 10, 200 and 600 µg/L, as confirmed by LC/UV. The water analysis indicates that within 48 hours adult zebrafish, but not embryos, transform BP-3 in part to benzophenone-1 (BP-1), an estrogenic compound also used as UV filter. BP-3 and BP-1 are accumulated in fish up to 21 µg/g and 266 ng/g, b.w. respectively. We analyse molecular effect by whole-genome transcriptomics (microarrays) and find no significant transcriptomic changes after 96 hours post fertilization at 312 µg/L BP-3. 123 transcripts are altered belonging to different pathways. By applying a target gene expression approach focusing on the endocrine system in the brain, liver and testis of adult zebrafish and in embryos, expression of estrogen receptor, androgen receptor, and vitellogenin are determined by qRT-PCR, as well as key enzymes involved in steroid hormone synthesis, such as the hydroxysteroid dehydrogenases, hydroxylases, aromatases. Transcript and protein level of vitellogenin is not induced up to 312 µg/L BP-3 in adult zebrafish (441 µg/L BP-3 in embryo). Our gene expression analysis data indicate that BP-3 has a low anti-estrogenic and anti-androgenic activity in adult zebrafish and embryos at environmentally relevant concentrations. The analytical chemical analysis emphasizes that effects may be linked to estrogenic activity and to anti-androgenic activity. Therefore, implementation of standard screening methods is expected by regulatory institutions and producers of chemicals.

MO 151
A comprehensive study on the toxicity of triphenyltin chloride to the rotifer Brachionus sp. at different biological organisation levels

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Endocrine disrupting chemicals (EDCs) exert effects at very low concentrations and can cause serious problems for the hormonale balance of various organisms. Exposure of wildtype to EDCs is not necessarily continuous, but may often occur in pulses. Consequently for the evaluation of the long-term effects on populations, it is essential to know whether such EDC-related effects are reversible. Three different substances selected for different modes of action were tested for their long-term impact on sex ratio, gonadal development, vitellogenin (VTG) induction and aromatotic activity in zebrafish; the androgen trenbolone binds directly and very effectively to the androgen receptor. Ethinylestradiol, a synthetic derivative of estradiol, causes feminization in wildlife and humans. The fungicide prochloraz acts as an aromatase inhibitor by direct interference with the aromatization of androgens to estrogens. All compounds have previously been shown to cause striking effects in zebrafish, but recovery has never been studied in detail in order to test whether EDC-related effects are reversible, an exposure scenario limited to 60 d was followed by (a) a recovery period of 40 d or (b) continued exposure for another 40 d. Four effects levels were examined: (1) population level: sex ratio; (2) organism level: growth; (3) organ/tissue level: histology of gonads (light microscopy); and (4) molecular level: vitellogenin induction (ELISA) and aromatase expression (RT-qPCR).

Results show clear correlation of effects at all levels, but also clear differences between the two different exposure groups. We conclude that endocrine disruption in zebrafish following discontinuous exposure is only partially reversible and may thus have serious implications for fish.

MO 152
Testing chemicals for endocrine activity by 21-day fish screening assay

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Beyond designed properties, plant production products, biocides, pharmaceuticals and other chemicals may pose undesired impacts on aquatic environment via sewage. Endocrine Disrupting Chemicals (hormone-like) are toxicants that cause various effects in regulatory functions depending on length of exposure period, age, sex and stage of development. Testing chemicals for estrogenic activity is required by law. For this reason, OECD Guideline for Testing of Chemicals No 230 (Sept 2009) introduced bioassay using endpoint biomarker - VTG (vitellogenin) in fish. Zebrafish WT AB adults of both sexes were exposed for 21 days to four chemicals: E2, prochloraz, flutamide and propiconazole. VTG biomarker of endocrine activity was determined in whole body homogenates by ELISA and additionally gonadosomatic index (GSI) was reported. Results were analysed by ANOVA statistics. Natural estrogen E2 caused increase of VTG in males and prochloraz (fungicide, aromatase inhibitor) decreased cause of VTG in females. Therefore, positive controls confirmed correct test design. Changes of VTG levels detected in female fish exposed to flutamide (nonsteroid anticanancer drug) suggest U shaped concentration-effect curve. Decrease of GSI in male fish was also observed. VTG levels were increased in female and decreased in male fish corresponding with concentration of propiconazole (biocide, fungicide) whereas GSI in male fish was increased.

Endocrine evaluation of activity in in vivo models requires integration of process in complex organism. Therefore, implementation of standard screening methods is expected by regulatory institution in industries and producers of chemicals.
Sexual endocrine disruption in fish with focus on estrogen receptor antagonists— a fish life cycle test with fulvestrant

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Phenyltin compounds in particular triphenyltin (TPT) are widely applied as effective biocides for different industrial and agricultural purposes. These compounds have been detected in the marine environment but little is known regarding their toxicity to the marine organisms. This study was designed to comprehensively investigate the molecular, histologic and endocrine responses of the rotifer, Brachionus sp., upon waterborne exposure to TPT chloride (TPTCl). Our results indicated that the population growth rate of the rotifers was decreased as TPTCl concentrations increased. A 96-h EC50 value for population growth inhibition was found to be 1.96 µg/L, which was ten times lower than the 96-h LC50 value derived from a standard acute toxicity test (i.e., 29.6 µg/L; 95% Cl: 27.2 - 32.1 µg/L). At molecular level, gene expressions of twelve heat shock proteins (hsp), four glutathione S-transferases (GST), two retinoic acid X receptor (RXR) and thirteen cytochrome P450 monoxygenases (CYP) were studied for their responses to TPTCl exposure. At 20 µg TPTCl, hsp 90α, GST and CYP 29 were significantly up-regulated with the relative expression levels to the control up to 32.9, 4.4 and 62.6 folds, respectively. Temporal trends of these three genes were further studied in rotifers exposed to TPTCl over a period of 24-h. The expressions of these genes showed an initial increase trend in the first few hours after exposure, peaking at 3 h (for hsp 90α and GST) and 12 h (for CYP 29) and then followed by a gradual decline. The overall findings of this study provide a better understanding on toxic mechanisms of TPT-mediated effects in the rotifer.

MO 153
Expression of all-trans and 9-cis retinoic acid receptor (RAR/RXR) genes during the zebrafish early development
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The zebrafish genome encompasses no less than 10 different genes coding either for retinoic acid (RAR) or 9-cis retinoic acid (RXR) receptors, which participate in the retinoic acid response as well as in many other hormone-dependent regulatory pathways. In this work we analysed the changes in mRNA abundance of each of these ten genes (RARαa, RARαb, RARγa, RARγb, RXRαα, RXRαβ, RXRβα, RXRββ, RXRγa, RXRγb) during the first 5 days of zebrafish development. The data shows a transition from maternal to embryonic transcripts during the first 48-48 h post fertilization, as well as a differential response of the receptor genes to the exogenous addition of tretinoin, particularly during the first 24 h after fertilization. These results suggest that this transitional period between maternal to embryonic mRNA is particularly sensitive to the presence of putative disruptors (retinoids or retinoids), and that the RAR/RXR system is functional throughout the zebrafish eutherioembryonic period. As several emerging contaminants are susceptible of disrupt either the RAR/RXR or other mechanistically related hormone regulatory systems (TR, CAR, PXR, PPAR, and others), the analysis of the retinoid/rexoid response in zebrafish embryos facilitates its implementation as convenient, non-animal model to assess these potentials risks.

MO 154
Sexual endocrine disruption in fish with focus on estrogen receptor antagonists— a fish life cycle test with fulvestrant
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Reproductive toxicity of methyltestosterone in embryo and adult zebrafish
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Expression of all-trans and 9-cis retinoic acid receptor (RAR/RXR) genes during the zebrafish early development
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Estrogen receptors represent important interfaces for mechanisms concerning gonadal development and reproduction of fish and therefore target sites for endocrine disruptors. Within the scope of establishing a tiered testing strategy to detect and assess a potential for sexual endocrine disruption in fish, data is needed to detect sensitive windows of exposure. Furthermore, the respective windows of effect manifestation should be identified. Especially for the estrogen receptor antagonists, a data gap is evident. A hsp 90α gene expression study was performed at the Fraunhofer IME to detect the population relevant effects of the selective estrogen receptor down regulator (SERD) fulvestrant on zebrafish. The nominal test concentrations were 1.3, 4.0, 13 and 40 µg/L. The test was performed under flow through conditions.

The test was initiated by introduction of fertilised eggs into the test vessels. The test fish were kept until they reached the adult stage. Survival, Growth and reproductive parameters were measured. Secondly, a filial generation was performed. The measured effects can be related to initial concentrations and to time weighted average concentrations during defined life stages.

There is still a data-poor situation for anti-estrogenic effects as testicular FSH receptor and steroidogenic enzymes were induced. There is still a data-poor situation for anti-estrogenic effects as testicular FSH receptor and steroidogenic enzymes were induced. The significant anti-estrogenic effects on survival and reproduction as sensitive window of manifestation for the estrogen modulated mode of actions.

A significant effect on reproduction could be observed at 13 and 40 µg fulvestrant/L. The cumulative number of fertilised eggs was found to be reduced. The results confirm findings from reproduction studies and the observed depression in reproduction as sensitive window of manifestation for the estrogen modulated mode of actions.

A reproduction test the DF50 in the water column was measured to be approximately 0.5 d. The only effect was a temporary one on fertilisation rate at 632 µg/L (initial concentration) on days 4 to 6. The flow-through test at concentrations of 40, 13, 4 and 1.3 µg/L will be ended by the end of 2011.

MO 156
Characterization of the mode of action of the pharmaceutical clotrimazole on testicular steroidogenesis in zebrafish
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Clotrimazole is a pharmaceutical fungicide that has been recently detected in aquatic environment. This substance is known to inhibit CYP enzymatic activities, including several CYP 3A4/5 genes involved in the metabolism of xenobiotics. However, little is known about endocrinology-disrupting properties of clotrimazole. In this study, we aimed to elucidate the potential mechanisms of clotrimazole disrupting endocrinology. In vitro testicular explants were exposed to similar concentrations of clotrimazole, and no effect was observed on transcript levels of steroidogenic enzymes. However, clotrimazole inhibited 11-KT release in the culture medium. This result suggests that clotrimazole does not act directly on testis to regulate the transcription activity of these genes. Induction of steroidogenetic genes could be interpreted as a compensatory biological response to inhibition of cytochrome P-450 dependent steroidogenetic enzymes.

To support this hypothesis, a network of functional genes of the pituitary-gonad axis was used. We showed that clotrimazole induce a cascade of molecular events in pituitary that modulates the activity of pituitary Gonadotropin releasing hormone receptors (GnRH-R) and folliculo- stimulating hormone (FSH) -subunit, as well as testicular FSH receptor and steroidogenetic enzymes were induced. All together, these molecular events are consistent with the involvement of FSH in inducing steroidogenetic gene expressions to compensate the inhibitory action of clotrimazole on 11-KT synthesis.

Our study highlights the relevance of studying a network of relevant genes of the pituitary-gonad axis to investigate the mode of action of clotrimazole on the gonadal system of fish. Such approach could be extended to other compounds acting as inhibitor of P450-steroidogenetic enzymes. The disruption of testicular steroidogenesis raises further concerns about the impact of clotrimazole on reproduction.

MO 157
Reproductive toxicity of methyltestosterone in embryo and adult zebrafish
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Reproductive toxicity of methyltestosterone in embryo and adult zebrafish
C. Rivero-Wendt1, M. Monteiro2, R. Oliveira2, J. Domingues3, A.M.Y.M. Soares4, C.K. Grisolia1

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Such approach could be extended to other compounds acting as inhibitor of P450-steroidogenetic enzymes. The disruption of testicular steroidogenesis raises further concerns about the impact of clotrimazole on reproduction.
**MO 158**
Sub-lethal effects of mixture of compounds in Zebrafish (Danio rerio)
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The prevalent use of different compounds in industry, agriculture, health care and other activities lead to an increased release of these compounds to the aquatic environments. Considering the risk of the mixture of compounds in these environments, it is unlikely that fish are exposed to one single substance each time. There are few studies to evaluate the sub-lethal effects of mixture of different compounds on development stages, molecular physiology, and behavior of fish. Zebrafish, Danio rerio, is a suitable model in fish embryo toxicity test (FET) and behavioral studies. To evaluate the toxicity of mixture of compounds, the zebrafish embryos were exposed to single substances, including ethinylestradiol, nonylphenol, benzo-a-Pyrene, beta-naphthoflavone, and silver. The embryonic sub-lethal endpoints were heart rate, pigmentation, and eye development. The lowest concentration of each single substance that might cause an effect (ECx) has been considered in joint mixture exposure. Since mixture concentration below the threshold, which can cause no effect, might impair fish behavior, the movement of zebrafish larvae after 6 dpf (days post fertilization) has been studied. In molecular physiological level the induction of vitellogenin and CYP1A, in protein and gene expression levels, were measured in adult fish exposed to single compound and joint mixture to demonstrate the endocrine disrupting effects. Due to the interaction between the compounds in the mixture, the estrogenic and xenobiotic activities in the multi-contaminated sediment extract, which has been shown to exert multiple toxicological activities as determined by a panel of in vitro bioassays. Both the crude extract and 4 fractions of increasing polarity (F1 to F4) and obtained from normal phase solid phase extraction were tested on the bioassays. Overall, crude and/or fractions were active on all endpoints, demonstrating the suitability of the bioassay to detect biologically active compounds. The crude extract, weak activities were detected in F1, F2 and F3 fractions whereas in vitro estrogenic activity was only detected in F2 and F3. F2 and F3 are known to contain estrogenic active compounds, i.e., BP1, THB, 4BP and 44’BP, as shown by their ability to induce GFP in a new developed in vitro and in vivo models. In vivo, three specific zebrafish reporter gene models expressing zebrafish estrogen receptors (ZfERs) in zebrafish hepatic cell line (ZFL) were used. Estradiol activity of BPs was quantified by estrogen receptor activity in zebrafish embryos and results showed a significant decrease in estrogenic activity in zebrafish embryos exposed to joint mixture of compounds. The results demonstrated that MT might induce endocrine disruption in exposed early-life stages of zebrafish. In the other hand, the adult males and females that already have the endocrine system completely developed might be able to cope with MT stress as evidenced by the higher HSI registered. The lower levels of Vtg registered in MT-exposed group could be related to the presence of MT in food, as well as the presence of other MT-inducing compounds in the environment.

**MO 159**
Zebrafish embryos as biological tools for detection of estrogenic and dioxin-like chemicals in environmental matrices
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Recent studies have reported the use of fish embryos as in vivo mechanism-based screening bioassays for the specific detection of biologically active compounds. In this study, we discuss the potential use of zebrafish embryos as a model for the detection of estrogenic and dioxin-like compounds in environmental matrices. Effect on phase I xenobiotic metabolism was assessed using EROD (Cyp1a-like) and BFCOD (Cyp3-like) assays in living embryos; estrogenic activity was measured using a transgenic zebrafish reporter gene expressing green fluorescence protein reporter gene under the control of the aromatase-B promoter (AroB-GFP). A panel of standards chemicals was first tested in order to calibrate the in vivo bioassays (AroB-GFP) and result showed that dioxin-like compounds were strongly active on the BFCOD assay. Then, these bioassays were applied to a multi-contaminated sediment extract, which has been shown to exert multiple toxicological activities as determined by a panel of in vitro bioassays. Both the crude extract and fractions of increasing polarity (F1 to F4) and obtained from normal phase solid phase extraction were tested on the bioassays. Overall, crude and/or fractions were active on all endpoints, demonstrating the suitability of the bioassay to detect biologically active compounds. The crude extract, weak activities were detected in F1, F2 and F3 fractions whereas in vitro estrogenic activity was only detected in F2 and F3. F2 and F3 are known to contain estrogenic compounds such as alkylphenols, parabens and steroids (data not shown). In contrast to the crude extract, no estrogenic activity could be detected in F1 fractions. The result showed that zebrafish embryos are sensitive and reliable tools to detect estrogenic and dioxin-like chemicals in environmental matrices and could serve as useful tools for effect-directed analysis approaches. Fundings: P189-ECOI and ITN EDA-EMERGE.

**MO 160**
Evaluation of estrogenic activities of benzenophene derivatives using innovative in vitro and in vivo zebrafish models
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Benzenophene derivatives (BP) are used as UV filters in many different products. These lipophilic compounds were recently described as ubiquitous contaminant of different consumer products and a potential endocrine disruptor. In this study, we assessed the estrogenic activity of BP in a model fish species, the zebrafish (Danio rerio), by combining newly developed in vitro and in vivo models. In vitro, three specific zebrafish reporter gene models expressing zebrafish estrogen receptors (ZfERs) in zebrafish hepatic cell line (ZFL) were used. Estradiol activity of BPs was quantified by estrogen receptor activity in zebrafish embryos and results showed a significant decrease in estrogenic activity in zebrafish embryos exposed to joint mixture of compounds. The results demonstrated that MT might induce endocrine disruption in exposed early-life stages of zebrafish. In the other hand, the adult males and females that already have the endocrine system completely developed might be able to cope with MT stress as evidenced by the higher HSI registered. The lower levels of Vtg registered in MT-exposed group could be related to the presence of MT in food, as well as the presence of other MT-inducing compounds in the environment.

**MO 161**
Endocrine disrupting effects of OSPW and ozonated OSPW on sex steroid synthesis and signaling in fathead minnows
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Ozonation attenuates affects of OSPW on some endocrine endpoints, and the effect is more prominent in males than in females. There is concern about the large volume of oil sands process-affected water (OSPW) produced by surface mining of the oil sands deposits in Alberta, Canada. OSPW has endocrine disrupting effects on exposed fish and in vitro. Although ozonation attenuates some of the endocrine disrupting effects of OSPW, the effects of ozone-treated OSPW on oestrogen and testosterone (T) metabolism and signaling in fathead minnow (Pimephales promelas) were measured in this study using a panel of standards chemicals, including ethinylestradiol, nonylphenol, benzo-a-Pyrene, beta-naphthoflavone, and silver. The embryonic sub-lethal endpoints were heart rate, pigmentation, and eye development. The lowest concentration of each single substance that might cause an effect (ECx) has been considered in joint mixture exposure. Since mixture concentration below the threshold, which can cause no effect, might impair fish behavior, the movement of zebrafish larvae after 6 dpf (days post fertilization) has been studied. In molecular physiological level the induction of vitellogenin and CYP1A, in protein and gene expression levels, were measured in adult fish exposed to single compound and joint mixture to demonstrate the endocrine disrupting effects. Due to the interaction between the compounds in the mixture, the estrogenic and xenobiotic activities in the multi-contaminated sediment extract, which has been shown to exert multiple toxicological activities as determined by a panel of in vitro bioassays. Both the crude extract and 4 fractions of increasing polarity (F1 to F4) and obtained from normal phase solid phase extraction were tested on the bioassays. Overall, crude and/or fractions were active on all endpoints, demonstrating the suitability of the bioassay to detect biologically active compounds. The crude extract, weak activities were detected in F1, F2 and F3 fractions whereas in vitro estrogenic activity was only detected in F2 and F3. F2 and F3 are known to contain estrogenic compounds such as alkylphenols, parabens and steroids (data not shown). Estradiol and BFCOD gave parallel results, with significant activities in crude, and F1/F2/F3 fractions. The crude extract and the in vivo bioassays show the suitability of zebrafish embryos-based assays to detect estrogenic and dioxin-like chemicals in environmental matrices and could serve as useful tools for effect-directed analysis approaches. Fundings: P189-ECOI and ITN EDA-EMERGE.

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**MO 162**
Differences in fathead minnow fecundity using tiles with and without trays for fish endocrine assays
Wildlife International Ltd, Easton, United States of America

Differences in fathead minnow fecundity using tiles with and without trays for fish endocrine assays
A trial to further evaluate the use of traws with designs for the FISH SHORT-TERM REPRODUCTION ASSAY WITH THE FATHEAD MINNOW (Pimephales promelas) (FSTA) test. The five-week trial simulated the experimental design of the FSTA test that has a two-week pre-exposure period followed by a three-week exposure period. No test substance was used in the trials. Fish were divided into two test groups, one with tiles only and the other with traws and tiles. The test compartments followed the guideline requirement of 15 eggs per female per reproductive day and have similar CV's. The data for weeks 3 to 5 of the trial simulates the exposure phase of the test. The tile only group had a mean fecundity of 19.66 eggs per female per day and a CV of 26.7%, while the tile and tray group had a mean of 27.95 eggs per female per day and a CV of 40%. The head sections, including the reproductive endocrine system was assessed by the gonad-somatic index (GSI), gonadal histology, plasma steroids (estrogen [E2] and testosterone [T]), 11-ketotestosterone [11-KT]), hydroxysteroid dehydrogenase [HSD], and plasma vitellogenin (VTG). Results indicated that the DE-71 exposure did not affect survival or body weight, but reduced male GSI. Reproductive function, gonad histology, and endocrinological measures of reproductive status were altered. Exposure to DE-71 did not alter plasma E2, a known oestrogen, to the observed levels unexposed to DE-71. The results of the trial were not in harmony with the expected effect on the gonadal differentiation demonstrating similar effects of polybrominated diphenyl ethers on the reproductive system of male fish. Evaluation of HSD activity and the male-only androgen 11-KT will be presented to describe whether the effect may be related to general alteration of early steroidsogenesis as measured by HSD, or if DE-71 is more specifically altering androgenesis in male fathead minnows as measured by 11-KT and testis, and acting in an anti-androgenic manner.

MO 164 Endocrine disruptive effects of the common feed contaminant 17α-trenbolone on the Australian Murray River rainbowfish (Melanotaenia fluviatilis) E.P. Kaps, N. V. Pettigrew, N. Ngugoda 1

1Fort Environmental Laboratories, Stillwater, United States of America

MO 165 Effects of trawls eluates on Vitellogenin levels and EROD activities in rainbow trout (Oncorhynchus mykiss) T. Nolte, P. Schättler, T. Stahl, J. Sturle University of Gothenburg, Gothenburg, Sweden

The consumption of textiles in the world is increasing every year. Several chemicals used in textile industry and fabrics may have adverse effects on aquatic organisms. Alkylphenols (APs) and their metabolites are of global concern (3T3-Fio in vivo induction). Therefore, in this study, we investigated the expression of different cluster of differentiation (CD) -receptors associated with T-cell maturation in a representative fish species. Phagocytosis is one of the crucial functions of the innate immune system, and is one of the rare, established biomarkers for the immune system in ecotoxicology. Intracellular hydroxysteroid dehydrogenase [HSD], and plasma vitellogenin (VTG). Results indicated that the DE-71 exposure did not affect survival or body weight, but reduced male GSI. Reproductive function, gonad histology, and endocrinological measures of reproductive status were altered. Exposure to DE-71 did not alter plasma E2, a known oestrogen, to the observed levels unexposed to DE-71. The results of the trial were not in harmony with the expected effect on the gonadal differentiation demonstrating similar effects of polybrominated diphenyl ethers on the reproductive system of male fish. Evaluation of HSD activity and the male-only androgen 11-KT will be presented to describe whether the effect may be related to general alteration of early steroidsogenesis as measured by HSD, or if DE-71 is more specifically altering androgenesis in male fathead minnows as measured by 11-KT and testis, and acting in an anti-androgenic manner.

MO 166 Effects of 17β-oestradiol on thymus volume and regionalisation in juvenile sea bass (D. labrax L.) E.H. Seemann, T. Monsinjon, T. Knigge, C. Minier Laboratory of Ecotoxicology- aquatic environment (LEMA), Le Havre cedex, France

Phagocytosis is one of the crucial functions of the innate immune system, and is one of the rare, established biomarkers for the immune system in ecotoxicology. Intracellular hydroxysteroid dehydrogenase [HSD], and plasma vitellogenin (VTG). Results indicated that the DE-71 exposure did not affect survival or body weight, but reduced male GSI. Reproductive function, gonad histology, and endocrinological measures of reproductive status were altered. Exposure to DE-71 did not alter plasma E2, a known oestrogen, to the observed levels unexposed to DE-71. The results of the trial were not in harmony with the expected effect on the gonadal differentiation demonstrating similar effects of polybrominated diphenyl ethers on the reproductive system of male fish. Evaluation of HSD activity and the male-only androgen 11-KT will be presented to describe whether the effect may be related to general alteration of early steroidsogenesis as measured by HSD, or if DE-71 is more specifically altering androgenesis in male fathead minnows as measured by 11-KT and testis, and acting in an anti-androgenic manner.

MO 167 17β-oestradiol impair s head kidney leucocyte phagocytosis in juvenile sea bass (D. labrax L.) E.H. Seemann, T. Monsinjon, S. Olivier, T. Knigge, C. Minier Laboratory of ecotoxicology- aquatic environment (LEMA), Le havre cedex, France

With the detection of oestrogen receptors in immune relevant organs and cells, it seems likely that the functionality of the immune system is also dependent on the concentration of sex hormones in the organism. Knowing that the concentrations of steroid hormones are elevated in the aquatic environment, the question of effects on the immune system of aquatic organisms arises. Exposure possibly leads to direct as well as indirect consequences on the mature immune system of adult fish. But more importantly, the development of the relevant immune receptors in immune relevant organs and cells, and it seems likely that the functionality of the immune system is also dependent on the concentration of sex hormones in the organism. Knowing that the concentrations of steroid hormones are elevated in the aquatic environment, the question of effects on the immune system of aquatic organisms arises. Exposure possibly leads to direct as well as indirect consequences on the mature immune system of adult fish. But more importantly, the development of the relevant immune receptors in immune relevant organs and cells, and it seems likely that the functionality of the immune system is also dependent on the concentration of sex hormones in the organism. Knowing that the concentrations of steroid hormones are elevated in the aquatic environment, the question of effects on the immune system of aquatic organisms arises. Exposure possibly leads to direct as well as indirect consequences on the mature immune system of adult fish. But more importantly, the development of the relevant immune receptors in immune relevant organs and cells, and it seems likely that the functionality of the immune system is also dependent on the concentration of sex hormones in the organism. Knowing that the concentrations of steroid hormones are elevated in the aquatic environment, the question of effects on the immune system of aquatic organisms arises. Exposure possibly leads to direct as well as indirect consequences on the mature immune system of adult fish. But more importantly, the development of the relevant immune receptors in immune relevant organs and cells, and it seems likely that the functionality of the immune system is also dependent on the concentration of sex hormones in the organism. Knowing that the concentrations of steroid hormones are elevated in the aquatic environment, the question of effects on the immune system of aquatic organisms arises. Exposure possibly leads to direct as well as indirect consequences on the mature immune system of adult fish. But more importantly, the development of the relevant immune receptors in immune relevant organs and cells, and it seems likely that the functionality of the immune system is also dependent on the concentration of sex hormones in the organism. Knowing that the concentrations of steroid hormones are elevated in the aquatic environment, the question of effects on the immune system of aquatic organisms arises. Exposure possibly leads to direct as well as indirect consequences on the mature immune system of adult fish. But more importantly, the development of the relevant immune receptors in immune relevant organs and cells, and it seems likely that the functionality of the immune system is also dependent on the concentration of sex hormones in the organism. Knowing that the concentrations of steroid hormones are elevated in the aquatic environment, the question of effects on the immune system of aquatic organisms arises. Exposure possibly leads to direct as well as indirect consequences on the mature immune system of adult fish. But more importantly, the development of the relevant immune receptors in immune relevant organs and cells, and it seems likely that the functionality of the immune system is also dependent on the concentration of sex hormones in the organism. Knowing that the concentrations of steroid hormones are elevated in the aquatic environment, the question of effects on the immune system of aquatic organisms arises. Exposure possibly leads to direct as well as indirect consequences on the mature immune system of adult fish. But more importantly, the development of the relevant immune receptors in immune relevant organs and cells, and it seems likely that the functionality of the immune system is also dependent on the concentration of sex hormones in the organism. Knowing that the concentrations of steroid hormones are elevated in the aquatic environment, the question of effects on the immune system of aquatic organisms arises. Exposure possibly leads to direct as well as indirect consequences on the mature immune system of adult fish. But more importantly, the development of the relevant immune receptors in immune relevant organs and cells, and it seems likely that the functionality of the immune system is also dependent on the concentration of sex hormones in the organism.
functions. Further these results indicate a possible impact on the recruitment of the European sea bass population, given that the European eel stocks, serving as nursery areas, suffer from higher levels of oestrogen pollution.

MO 168

A non-invasive method based on head morphology to sex mature three-spined Stickleback (Gasterosteus aculeatus) in rearing conditions


Institut Vernouillet-halte, France

The three-spine stickleback (Gasterosteus aculeatus) is a small-bodied teleost fish which is a major component of shallow water food webs in the northern hemisphere. G. aculeatus is a reference species for experimental studies, in particular for the assessment of endocrine disruptors. Sex ratio can bias the outcomes of an experimental study, especially if this study focuses on reproduction or behaviour. Moreover, sex-ratio, by itself, can be a very relevant endpoint in experimental tests. A mathematical model to distinguish mature female and male three-spined Sticklebacks G. aculeatus is proposed. This method is based on sexual dimorphism in the head morphology.

The discrimination was established on five distances of interest on the head, divided by the standard length of fish. The parameters were estimated based on a training set composed of a database characterised by anatomical sex ratio and validated on a test set composed of 69 fish. Our model permits to balance between the percentage of fish that can be sexed and the percentage of fish correctly sexed. Compared to other available methods to sex G. aculeatus, our model is non-invasive, not expensive, rapid, and reliable, can be calibrated out of the breeding period.

MO 169

Effects of produced water from an offshore oil-platform; an in vivo study using the three-spined stickleback (Gasterosteus aculeatus) as a model species

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There is growing concern over the high levels of anthropogenic contaminants entering the Arctic region. In light of the anticipated expansion of oil exploration and production in Arctic areas, of particular concern is the potential impact of hydrocarbon-related contaminants on the Arctic ecosystems. Several of these compounds are known to exhibit endocrine disrupting properties. The predicted expansion of oil production into Arctic areas would pose a considerable environmental risk, and highlights the need for the implementation of robust indicator species for future biomonitoring programs.

The three-spined stickleback (Gasterosteus aculeatus) is a small teleost fish widely distributed throughout the Northern hemisphere, occurring as far north as Svalbard. This ubiquitous species is increasingly being recognized as an emerging model in ecotoxicology, notably as a sentinel for endocrine disruption. The stickleback can be used as a combined biomarker of both estrogenic and (anti-) androgenic compounds, and with the sequencing of its genome a number of molecular tools are being developed for the assessment of contaminant exposure. The stickleback has the potential of becoming a valuable indicator species to identify and monitor the impact of anthropogenic pollutants in Arctic ecosystems, and be included in future biomonitoring programs.

The objective of this study was to evaluate the impact of stress tolerance in fish after exposure to produced water, using the three-spined stickleback as a model organism. Effects were assessed by quantification of gene expressions (UDP-G, CYP1A, VTG, PLA), in addition to measuring the levels of glucose and cortisol.

MO 170

ZnO nanoparticles exposure alters transcriptomic profile in Hydra

S. Yum, A. Lee, H. Won, S. Woo

Korea Ocean Research and Development Institute, Geoj, South-Korea (Rep)
The acute toxicity test for 20 nm ZnO and 100 nm ZnO and comparative transcriptomic profile analysis using Hydra exposed to 20 nm ZnO and 100 nm ZnO were carried out. As a result, the LC50 for 22 hours was 8.7 mg/L in 20 nm ZnO exposure and 14.9 mg/L in 100 nm ZnO exposure group. For the microarray experiment we exposed Hydra to 20 nm ZnO and 100 nm ZnO as the concentration of 1/50 of LC50 for 12 hours and hybridized those RNAs extracted from the exposed groups with that of control group. As a result, 237 genes were induced and 137 genes were induced in 10 fold in 20 nm ZnO exposure. Among them the expression of mulitdrug Resistance like Protein 1 CG6214-FM gene increased over 10 fold. In 100 nm ZnO exposure, the expression of 137 genes were induced over 10 fold and 106 genes were reduced over 10 fold. The upregulated more than 2-fold in both 20 nm and 100 nm ZnO exposures were 886 genes and more than 2 fold in 20 nm ZnO exposure, were 1631 genes. In the bioaccumulation and sublethal toxicity of 4-NP by exposing first to four meter choramin larveus (Chromis riparius) to a 4-NP concentrations of 6.71 and 184.6 mg/kg d. wt, 4-NP in pore-water was monitored and the expression of 131 genes were expressed by ZnO nanoparticles exposure showed that those nanoparticles tend to affect the transcription of genes in cellular processes and signaling group in most.

MO 171

Bioaccumulation and sublethal toxicity of 4-nonylphenol in chironomus riparius meigen 1804 (Diptera, chironomidae) larvae: assessment of mentum shape variations by means of morphometric

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In this work, we studied bioavailability, bioaccumulation and sublethal toxicity of 4-NP by exposing first to four meter choramin larveus (Chromis riparius) to a 4-NP concentrations of 6.71 and 184.6 mg/kg d. wt, 4-NP in pore-water was monitored and the expression of 131 genes were expressed by ZnO nanoparticles exposure showed that those nanoparticles tend to affect the transcription of genes in cellular processes and signaling group in most.

Endocrine disrupting compounds (ECDs) are known to cause intersex and altered sex ratios in fish but effects of endocrine disruptors on bivalve molluscs have only been reported for few estrogens and androgens in Enodrina, it is a very sensitive species for studying the effects of the chemical pollutants on the bivalve molluscs. The 4-nonylphenol ethoxylates are surfactants widely used to produce oil-soluble detergents and emulsifiers. In the environment these substances are transformed by microorganisms together with heavy metals which are involved in the formation of the tertiary structure of the ECDs. The study of the effects of 4-nonylphenol ethoxylates on the reproduction of the bivalve molluscs needs to take into account not only the direct effects of the hormone, but also the indirect effects of heavy metals.

Yolk-like protein levels in bivalve molluscs have so far been determined by an alkali-labile phosphate (ALP) measurement assay, which is an indirect method for detection of yolk-like proteins in bivalves is therefore highly needed and we started development of an ELISA (Enzyme-Linked Immunosorbent Assay) for yolk protein in bivalve species. Yolk protein levels in different bivalve tissues were determined both by ALP and the developed ELISA and a comparison of results obtained from both methods was made. In addition, river water russels (Unio tumidus) were exposed to E2 (57, 164 and 512 ng/L) for seven weeks during the reproductive period to investigate if intersex could be induced. Histological examination of the gonads revealed that intersex could not be induced at these E2 concentrations and the presence and quantity of yolk protein in tissue and hemolymph was investigated.

MO 173

Comparison of var sexs and penis development between the rock shell, Thais clavigera (Muricidae) and the ivory shell, Babylonia japonica (Buccinidae)


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Endocrine disrupting compounds (ECDs) are known to cause intersex and altered sex ratios in fish but effects of endocrine disruptors on bivalve molluscs have only been reported for few estrogens and androgens in Enodrina, it is a very sensitive species for studying the effects of the chemical pollutants on the bivalve molluscs. The 4-nonylphenol ethoxylates are surfactants widely used to produce oil-soluble detergents and emulsifiers. In the environment these substances are transformed by microorganisms together with heavy metals which are involved in the formation of the tertiary structure of the ECDs. The study of the effects of 4-nonylphenol ethoxylates on the reproduction of the bivalve molluscs needs to take into account not only the direct effects of the hormone, but also the indirect effects of heavy metals.

The characteristics of the development of male genitalia (penis and vas deferens) in imposex-exhibiting female and male rock shells, Thais clavigera (Muricidae), were histologically examined using specimens from wild populations and tributyltin (TBT)-exposed females in the laboratory. Vas deferens and penis development was already observed in both imposex-exhibiting female and male rocks shells even at approximately 6 months old. A variety of vas deformers morphogenetic patterns were observed in wild female T. clavigera. This was summarised. The immature vas deferens at an initial stage, however, was only observed beneath or behind the penis, and the vas deferens (i.e., vela) of the capsule gland in TBT-exposed fishes was different from the characteristics of vas deformers formation observed in wild females. Taking into consideration both the observed results from wild female specimens and from TBT-exposed females in the laboratory, the vas deferens sequence (VDS) index for T. clavigera was proposed as VDS 1-6. Meanwhile, we also histologically examined development of genitalia in the ivory shell, Babylonia japonica (Buccinidae), using 2-year-old shells from wild populations and laboratory-reared juveniles for 0-20 months of age. Differentiation of gonad (i.e., ovary) was initiated before 16 months of age, whereas differentiation of penis was initiated only after 18 months of age, and progression of differentiation was slower in males than in females. However, vas deferens differentiation was observed in both sexes at 14 months of age, although no penis was observed in them. Forms of penis was recognized in almost all males at 16 months of age, although vas deferens was not yet completely developed. Vagina, bursa copulatrix and capsule gland were developing in 14-month-old females. Albumen gland and receptaculum seminis were also developing in 16-month-old females. Differentiation of development of genitalia in the ivory shell, suggesting that regulatory mechanism of reproduction might be different between mollusks (prosobranch gastropods) and vertebrates. Moreover, there might be a considerable degree of difference on vas deferens and penis development
between the rock shell, *T. clavigera* (Mureidae) and the ivory shell, *R. japonica* (Buccinidae), namely, even among prosobranch gastropod species.

**MO 174**

Impact of Roundup® and 17α-Ethinylestradiol exposure on the liver morphology of bullfrog's tadpoles (Lithobates catesbeianus)

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Ectothermic vertebrates have melanocytes on several organs (lung, heart, thymus, and gonads) and tissues (mucous and connective tissues surrounding blood vessels), constituting an extracellular pigmentary system. Our results showed that both Roundup® and 17α-Ethinylestradiol provoked an increase in the liver pigmentation due to the presence of melanomacrophages (MMG) when compared to their respective controls. The melanomacrophages are pigment cells with macrophagic activity. They are found in hematopoietic organs and in even the liver of amphibians and have different types of granules in the cytoplasm, besides melanin, that suggest the presence of chemically distinct substances. The functional role of these viscer al melanocytes is unknown, but studies in low vertebrates like fishes and also in frogs showed that the melanomacrophages may be involved in inflammatory responses to bacterial infections and in the regulation of the immune reaction. These cells are present in the liver of the adult amphibians under normal conditions, but present a considerable increase under toxic conditions. This process is so drastic that the change on liver pigmentation due to these is the first to assess the liver response against inflammation. They do not only increase in number, but also form melanomacrophages aggregates (MMG). MMG are supposed to be involved in the inflammatory responses to low molecular weight biotic and abiotic substances in vertebrates. The mammalian macrophages, the frog melanomacrophages also accumulate hemoglobin by destruction of senescent erythrocytes. In this manner, the increase of the melanomacrophages and MMG in bullfrog tadpole exposed to Roundup® and 17α-Ethinylestradiol in the livers demonstrate a response to an inflammatory condition, which may jeopardize the development of the individual if in natural conditions the exposure to these xenobiotics are constant and maintained.

**MO 175**

Validation of the amphibian metamorphosis assay for potential endocrine disrupting chemicals with Xenopus laevis

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The test was chosen for the protection of aquatic species. Proliferation (PPR) 1107/2009 identifies the need to consider whether a substance is a potential endocrine disrupter in aquatic non-target organisms and the current draft of the PPR requirements refers to three screening assays for ecotoxicological endocrine-disrupting potential. Of these, we consider that our results are similar to the ring test results published by the OECD (Series on Testing and Assessment Document Number 77) and make a number of observations on methodology that may improve the reproducibility of these assays.

**MO 176**

Progestins - potent endocrine disrupters of the female reproductive system

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Progestrone signaling has received very little attention as a potential target for disruption although it is a key regulatory pathway in the development and function of the female reproductive system. Further research by the environment concerns the threat to egg-laying in wild fish. Progestins are extensively used in contraceptives and in other hormonal therapies in human and veterinary medicine. More information on the targets and effects of progestins in adult and early life stages is needed to assess the environmental risks of this type of compounds. The major objective of this study was to determine developmental and reproductive toxicities, assessing the impact of low environmentally relevant levels of each on embryo-fetal development. Using the Xiglu model (a zebrafish model) the effects of the cadmium in adults of Rana ridibunda showed the same results obtained here [3]. We also observed invasion of abundant immunity cells among the hepatocytes in the exposed to Roundup® and 17α-Ethinylestradiol in the livers demonstrate a response to an inflammatory condition, which may jeopardize the development of the individual if in natural conditions the exposure to these xenobiotics are constant and maintained.

**MO 177**

Biological identification of PBDE-47 by human liver microsomes and Cytochrome P450s and formation of potentially toxic metabolites

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During the past decade both animal and human studies have supported an association between polybrominated diphenyl ether (PBDE) flame retardants and neurobehavioral / neurodevelopmental disorders, particularly following in utero and postnatal exposure. Evidence is also growing suggesting that bioactivation by oxidative metabolism adds considerably to the toxic potential of PBDEs. Thus, there is a critical need to further our understanding of PBDE metabolism in humans. This study conducted a qualitative and quantitative characterization of the in vitro metabolism of 2,2',4',4'-tetrabromodiphenyl ether (BDE-47), the most abundant congener retained in humans, using recombinant human cytochrome P-450s (CYPs). BDE-47 (0.1 mM) was incubated with human liver microsomes (0.1 to 10 µM BDE-47). Of the 11 human CYPs that were screened, CYP2B6 was the predominant CYP capable of forming six different mono-hydroxylated-BDEs (OH-BDEs), s, including 3-OH-BDE-47, 5-OH-BDE-47, 6-OH-BDE-47, 2-OH-BDE-66, 4-OH-BDE-42, and 4’-OH-BDE-49. With the exception of 2’-OH-BDE-66, all of these metabolites have also been detected in human blood samples. The results of the in vitro bio-transformation of BDE-47, moreover, the analysis by full scan GC/MS of the metabolites of BDE-47 formed by CYP2B6 showed the formation of di-OH-BDE-47 and di-OH-dioxin, a novel metabolite. Kinetic studies of BDE-47 metabolism by CYP2B6 and pooled human liver microsomes found Km values ranging from 1-2 µM, indicating the high affinity of CYP2B6 for the formation of hydroxylated metabolites. The promiscuous role of CYP2B6 in the metabolism of BDE-47 will be important for future mechanistic and epidemiological studies investigating the potential of BDE-47 and its’ metabolites to produce neurobehavioral / neurodevelopmental disorders.

**MO 178**

Effect of DCCs on fish communities in the Leuvu River, Limpopo province, South Africa

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Communities present within aquatic ecosystems worldwide are the result of agricultural applications, urban development and industrial effluents. Current contamination by DDT in the Luvuvhu River, Limpopo province, South Africa is primarily due to IRS (indoor residual spraying) for mosquito vector control of the malarial epidemic. Moreover, the analysis by full scan GC/MS of the metabolites of BDE-47 formed by CYP2B6 showed the formation of di-OH-BDE-47 and di-OH-dioxin, a novel metabolite. Kinetic studies of BDE-47 metabolism by CYP2B6 and pooled human liver microsomes found Km values ranging from 1-2 µM, indicating the high affinity of CYP2B6 for the formation of hydroxylated metabolites. The promiscuous role of CYP2B6 in the metabolism of BDE-47 will be important for future mechanistic and epidemiological studies investigating the potential of BDE-47 and its’ metabolites to produce neurobehavioral / neurodevelopmental disorders.

*SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting*
MO 179
Distribution of endocrine disrupting pollutants in the Long Island Sound
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Water waste treatment facilities (WWTFs) input fresh water, nutrients, and contaminants to the Long Island Sound (LIS). While nutrient loads and concentrations for some contaminants are lower, there are many emerging contaminants, however there are numerous emerging contaminants, with known endocrine disrupting and bioaccumulating properties. Given the full range of toxic effects are still to be determined, and the regulatory framework controlling their use and allowable inputs into coastal waters has yet to be established. The water and sediment of the LIS are routinely sampled for concentration data of classical persistent organic pollutants, however there are numerous emerging contaminants, with known endocrine disrupting and bioaccumulating properties. Testing for these emerging contaminants is important to understand the inputs of emerging contaminant throughout the LIS, in order to determine the potential impacts on this estuarine ecosystem. Eight waste treatment facilities (WWTF) which discharge directly into the LIS were tested for different classes of known endocrine disrupting compounds (EDCs); perfluorinated compounds (PFCs), phthalates esters (PAEs), nonylphenol, octylphenol and bisphenol A. The steroidal estrogenic hormones and sulfated EDCs had generally low concentrations, yet partitioning of drugs between these compartments is limited. WWTFs are a known point source of these pollutants; research indicates that biodegradation during secondary treatment can increase concentrations of PFCs in wastewater effluent. The distribution of several of the target compounds between the suspended particulate and dissolved phases in the effluent waters was determined to be a function of ionic strength. The fraction of biologically active (normalized) data indicate that partitioning to dissolve organic matter (DOM) is important as a third phase for several target pollutants. The fate and distribution of target compounds entering the saline estuary from the effluent was further investigated; an important factor in wastewater distribution of these target compounds is the scavenging by particulates which increases as a function of ionic strength. The water-sediment distributions of target EDCs in different salinity and sediment quality regions of the LIS are presented. These results are an important first step in the investigation into the role of wastewater organic matter in the transport and distribution of target EDCs in an estuary.

MO 180
Assessment of intersex severity in grey mullets from three Portuguese estuaries - preliminary data
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All over the world, plentiful chemicals reach continuously the environment, and some of them are endocrine disrupters compounds (CDEs) which can cause diverse harmful effects in wild fish (also under low concentrations) including intersex condition, namely ovotestis (ovocytes in the testis), but can also affect human health.

The presence of intersex condition in ichthyofauna has been reported in numerous countries, including (scarce information) in Portugal, but no data was published on a large sampling and covering several estuaries, that have yet to be detected in this economically important region. It is critical to importatant to investigate the inputs of emerging contaminant throughout the LIS, in order to determine the potential impacts on this estuarine ecosystem. Eight waste treatment facilities (WWTF) which discharge directly into the LIS were tested for different classes of known endocrine disrupting compounds (EDCs); perfluorinated compounds (PFCs), phthalates esters (PAEs), nonylphenol, octylphenol and bisphenol A. The steroidal estrogenic hormones and sulfated EDCs had generally low concentrations, yet partitioning of drugs between these compartments is limited. WWTFs are a known point source of these pollutants; research indicates that biodegradation during secondary treatment can increase concentrations of PFCs in wastewater effluent. The distribution of several of the target compounds between the suspended particulate and dissolved phases in the effluent waters was determined to be a function of ionic strength. The fraction of biologically active (normalized) data indicate that partitioning to dissolve organic matter (DOM) is important as a third phase for several target pollutants. The fate and distribution of target compounds entering the saline estuary from the effluent was further investigated; an important factor in wastewater distribution of these target compounds is the scavenging by particulates which increases as a function of ionic strength. The water-sediment distributions of target EDCs in different salinity and sediment quality regions of the LIS are presented. These results are an important first step in the investigation into the role of wastewater organic matter in the transport and distribution of target EDCs in an estuary.

MO 181
Mothers living in two locations in the Philippines were obtained in 2008 and analyzed for three groups of organohalogen compounds (PCBs, PBDEs and HBCDs). PCBs were

specific accumulation of polychlorinated biphenyls and brominated flame retardants in human breast milk and scalp hair from the Philippines: levels, distribution and profiles
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The Mediterranean basin is one of the most vulnerable regions of the world to the global change due their climatic conditions characterized by summer drought and their high population density in these zones, are the principals arguments to evaluate the quality of these rivers.

The objective of this work was the determination of endocrine disruptors and related compounds belonging to different groups of chemical substances (10 estrogens, natural and synthetic, in free and conjugated form, 8 alkylphenols, 4 antiseptics, 2 antibacterials, 3 flame retardants, 2 anticoagulants and BPA) in four representative watersheds in Spain: Llobregat, Ebro, Júcar, and Guadalquivir.

In this study, a multiresidue analytical method for the analysis of water samples was applied, using the Thermo Scientific Equan ICM-MS system, an automated online preconcentration system. For the analysis of sediment samples a preconcentration procedure (PLE) was carried out using an automated ASE 200 system (Dionex, Sunnyvale, CA, USA), the solvent extraction was methanol:acetonitrile (1:1) mixture, for clean-up a solid phase extraction (SPE) was performed. A Thermo Scientific TSQ Vantage triple quadrupole mass spectrometer with an ELSD source was used for the MS analysis.

The assessment of the occurrence and occurrence of target compounds, although strong correlation of different compounds detected varied considerably depending on the sampling point. For example, in the case of water samples the concentrations of different compounds detected varied considerably depending on the sampling point. For example, in the case of water samples the concentrations of different compounds detected varied considerably depending on the sampling point. For example, in the case of water samples the concentrations of different compounds detected varied considerably depending on the sampling point. For example, in the case of water samples the concentrations of different compounds detected varied considerably depending on the sampling point. For example, in the case of water samples the concentrations of different compounds detected varied considerably depending on the sampling point. For example, in the case of water samples the concentrations of different compounds detected varied considerably depending on the sampling point.

In order to estimate the estrogrenic activities of samples, the EEQ were calculated using relative estrogenic potency (relative to 17β-estradiol). As it was expected, the results showed that estrogen activity, especially estradiol, were the largest contributors to the estrogenic potential with values between nd-4.60 ng/L. However the contribution of other compounds such as alkylphenols, BPA and anticoagulants to the total estrogenicity was low and ranged between nd-0.09 ng/L.

MO 183
Analysis and occurrence of endocrine disrupting compounds and related compounds in surface waters and sediments in rivers of the Iberian Peninsula
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Specific accumulation of polychlorinated biphenyls and brominated flame retardants in human breast milk and scalp hair from the Philippines: levels, distribution and profiles
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Due to the lack of adequate treatment facilities, large amount of municipal waste are directly dumped at dumping sites in the Philippines without proper management, probably causing several adverse environmental consequences and increased human health risk to local communities. Overall, the data on human exposure to organohalomgen contaminants is rather scarce and comprehensible studies are not available. The present study was therefore carried out to determine the concentrations of three group of persistent organohalogenals such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) in human breast milk and scalp hair samples from mothers living in two locations in the Philippines, viz., Payatas, a waste dumpsite, and Malate, a non-dumpsite. In addition, the present study examined relationships between contaminant levels in milk/hair samples. Human breast milk and scalp hair samples (n=30) from primiparae and multipara mothers living in two locations in the Philippines were obtained in 2008 and analyzed for three groups of organohalomgen compounds (PCBs, PBDEs and HBCDs). PCBs were
A. Fournier, R.C. Biever, L.E. Sayers, M.A. Cafarella

Smithers Viscarm, Wareham, United States of America

Plastics, vitellogenin concentration is an important biomarker and is a data point collected in the OECD 229, 230 and OPPTS 890.1350 test guidelines used to screen chemicals for potential endocrine activity in sexually mature male and spawning female fathead minnows (Pimephales promelas). Plasma vitellogenin concentration is also an important endpoint in the OECD 234 test for maturation of freshwater fish. Due to the inherent variability observed within a typical population of fish, examining historical control ranges is very important. The objective of this method is to determine historical control ranges of vitellogenin concentrations in plasma and to provide recommendations for considering a sample result to be an outlier. The outlier samples from female fish are typically characterized by low vitellogenin concentrations in water, sediment and fish in the DanShui River, Taiwan. Sampling was conducted every one and a half months in nine months. The concentrations in fish organs were also determined to estimate the organ distribution and the bioaccumulation of these chemicals. Water, sediment and fish (Oreochromis mossambicus and Oreochromis niloticus) were sampled from DanShui River. Water samples were pretreated with automated solid-phase extraction. Sediment and biota were extracted with matrix solid-phase dispersion. The quantification was done with ultra-high-performance liquid chromatography/tandem mass spectrometry and isotope dilution techniques.

Alkylphenolic substances and beta estradiol and bisphenol A in water, sediment and fish from Dan-Shui River, Taiwan

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1Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan

This study investigated the determination of feminizing chemicals including 4-tert-octylphenol (OP), 4-nonylphenol (NP), nonylphenol monoethoxycarboxylate (NP-EO), nonylphenol diethoxycarboxylate (NP-2EO), nonylphenol trineopthoxyacetate (NP-3EO), 4-tert-octyl estradiol, 4-tert-octyl estradiol and 4-tert-octyl ethinyl estradiol in river water, sediment and fish in the Dan-Shui River, Taiwan. Tainting, produced every one and a half months in nine months. The concentrations in fish organs were also determined to estimate the organ distribution and the bioaccumulation of these chemicals. Water, sediment and fish (Oreochromis mossambicus and Oreochromis niloticus) were sampled from DanShui River. Water samples were pretreated with automated solid-phase extraction. Sediment and biota were extracted with matrix solid-phase dispersion. The quantification was done with ultra-high-performance liquid chromatography/tandem mass spectrometry and isotope dilution techniques.

MO 185

Differentiating outliers in fathead minnow (Pimephales promelas) plasma vitellogenin concentration and their correlation to other assay endpoints

A.E. Fournier, R.C. Biever, L.E. Sayers, M.A. Cafarella

Smithers Viscarm, Wareham, United States of America

Plastics, vitellogenin concentration is an important biomarker and is a data point collected in the OECD 229, 230 and OPPTS 890.1350 test guidelines used to screen chemicals for potential endocrine activity in sexually mature male and spawning female fathead minnows (Pimephales promelas). Plasma vitellogenin concentration is also an important endpoint in the OECD 234 test for maturation of freshwater fish. Due to the inherent variability observed within a typical population of fish, examining historical control ranges is very important. The objective of this method is to determine historical control ranges of vitellogenin concentrations in plasma and to provide recommendations for considering a sample result to be an outlier. The outlier samples from female fish are typically characterized by low vitellogenin concentrations in water, sediment and fish in the Dan-Shui River, Taiwan. Sampling was conducted every one and a half months in nine months. The concentrations in fish organs were also determined to estimate the organ distribution and the bioaccumulation of these chemicals. Water, sediment and fish (Oreochromis mossambicus and Oreochromis niloticus) were sampled from DanShui River. Water samples were pretreated with automated solid-phase extraction. Sediment and biota were extracted with matrix solid-phase dispersion. The quantification was done with ultra-high-performance liquid chromatography/tandem mass spectrometry and isotope dilution techniques.

MO 186

Application of ultra performance liquid chromatography-tandem mass spectrometry for the determination of steroid estrogens and non-ionic surfactants in wastewaters

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Separation and determination of alkylphenol ethoxylates by using a triple quadrupole mass spectrometry method was developed and validated for the determination of steroid estrogens and alkylphenol ethoxylates in wastewater matrices. To date, analytical methods established in the literature for 17α-ethinylestradiol have been unable to achieve the respective proposed predicted no effect concentration of 0.1 ng l⁻¹. The estrogen analytical method enabled 17β-estradiol to be determined below the PNEC with a method detection limit of 0.06 ng l⁻¹ which has been validated in real environmental matrices. During the validation process, a trickling filter wastewater treatment works was monitored to demonstrate the methods application. Estrogen removal across the filters demonstrated good removals of natural free estrogens (≥62.0 %) with lower removals of synthetic 17α-ethinylestradiol (29.2 %) from wastewaters at 10 °C. The methods application illustrates the proposed methods capability to detect estrogens below PNEC values in real samples. Furthermore, a complete process mass balance for 17α-ethinylestradiol is now attainable which has previously posed a challenge due to the low environmental concentration typically exhibited, but determined as a result of the use of the proposed new methods. The analytical methods. The ultra performance liquid chromatography method developed for alkylphenol polyethoxylates enables these compounds to be determined in the ng l⁻¹ range which is crucial due to the reduced concentrations in crude sewage experienced over recent years.
In 2008 The European Chemicals Agency identified HBCDD as 1 of 14 substances of "Very High Concern", and in September 2010 HBCDD was added to REACH's Authorization List. In February 2011 HBCDD was selected to be phased out by EU REACH Regulation before 2015, if authorization is not granted.

In October 2011 POP Review Committee under the Stockholm Convention has assessed the risks from HBCDD, and it was concluded that HBCDD fulfills the criteria of a persistent organic pollutant (POP). This presentation will provide a succint up-to-date overview of HBCDD's properties, uses, regulation and discusses the risks associated with its prevalence in buildings, our homes and immediate environment.

MO 189
Statistical models for sex ratio data
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Studies of Fish sexual Development Test (FSTD) Test Guideline 234 call for 50% male and 50% female fish during the sex labile period to be placed in each replicate tank in the control and test concentrations at the start of the experiment, with the primary goal to determine whether the test substance causes sex reversal. The observed percent maximum variance when the true percent is 50% is 30%. Thus, all analyses of sex-ratio must deal with high variability. This is quite different from typical survival analysis, where control survival is close to 100%.

This variability affects both ECx and NOEC analyses. For NOECs, there is reduced power to detect an effect. With regression, there are increased model uncertainty and width of confidence intervals for ECx. One idea to reduce variability is to "normalize" sex-ratio by dividing each replicate response by the control mean response. Normalized responses are more variable, since they are ratios of two random variables. Also, such data are no longer independent, since they all have the same random variable denominator, thereby violating a basic requirement of regression and hypothesis testing models. Mathematical details show that the correlations among the normalized responses are not trivial and cannot be ignored.

The results of models and results of ECx estimates based on non-normalized responses were investigated using data from numerous studies done following TG234. Numerous well-accepted regression models for toxicity data were fit to the data, goodness-of-fit was assessed, and confidence intervals for ECx estimates for x=5, 10, 20, and 50 were obtained. Several hypothesis tests were used to obtain NOECs. These methods were compared to one another and to the regression results. Both regression and hypothesis test results s were supplemented by computer simulations that showed the conclusions are true in general.

The results show that for fish sex-ratio data based on phenotypic sex, regression models often fail to provide meaningful estimates of ECx for x=20 and sometimes for larger values of x. This is true regardless for any practical experimental design for the FSTD. The step-down Jonckheere-Terpstra test usually provides meaningful NOEC values that are useful for risk assessment. When genetic sex is known, so true sexual reversal can be measured, the situation is quite different and regression models are very useful.

MO 190
Progress of assessment under the Japanese program on endocrine disruption: EXTEND2010
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Ministry of the Environment, Japan implemented their third research program on endocrine disruption titled "EXTEND2010"(EXTEND: Extended Tasks on Endocrine Disruption) in July 2010. Target of the new five-year program is to accelerate hazard assessment on endocrine disruption of chemical substances, prioritizing organisms in the environment, and to consequently conduct risk assessment to see whether any regulatory risk management measures should be introduced.

Test protocols of fish, amphibians and invertebrates have been developed through bilateral or multilateral collaborations. In vitro assays using receptors of fish, amphibians and invertebrates are also being developed in the ministry’s program. Two-tiered framework for assessing endocrine disrupting effects to organisms in the environment is being developed. This framework is designed to effectively identify potential candidates for endocrine disruptors using available information and test results. Reliability evaluation of all candidates using OECD candidate criteria should be relevant to be selected candidates subject to testing to assess their endocrine disrupting effects to aquatic organisms. The existing knowledge is re-evaluated to identify which in vitro assays should be conducted for prioritization to select candidate chemicals for in vivo tests. After the two batches of reliability evaluation twenty-five chemicals were identified as candidates for testing. In vitro assays were conducted for eighteen chemicals in FY 2010 and 2011 and estrogenic and anti-estrogenic activities were detected in some of them. The way of prioritized selection of chemicals subject to tier 1 in vitro testing is being discussed based on the results.

Progress and updated status of the assessment of chemicals under the EXTEND2010 will be demonstrated.

MO 191
Impact of animal manure separation technologies on steroid hormone distribution - consequences for agricultural practices
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When steroid hormones are emitted into the environment, they may have harmful effects on the reproduction system of aquatic life. Until now, research has primarily focused on human excretion, demonstrating that steroid hormones reach the aquatic environment due to insufficient removal in waste water treatment processes. However more recently, it has been revealed that agricultural practices also may add to the environmental burden of steroid hormones. So far, research activities have mainly focused on steroid estrogens, but also androgens, progesteragens and glucocorticoids, expressed in the vertebrate steroidogenesis, may occur at substantial levels in animal manure and should be addressed.

In agricultural practices the animal manure can be applied to the soil as raw manure, but also as a solid or liquid manure fraction, since current livestock production facilities utilizes a recently developed technology, which separates raw animal manure into a solid and a liquid fraction. This technology offers an improved handling and refined distribution of the manure nutrients to the farmlands and the possibility to reduce the environmental impact of manure nutrients, especially avoiding the surplus load of phosphorous.

In the present work we investigated the distribution of steroid hormones (pregnenolone, progesterone, dehydroepiandrosterone, androstenedione, testosterone, dihydrotestosterone, estrone, 17α-estradiol and 17β-estradiol) in raw manure and manure separates from 10 to 15 different pig farms in Denmark utilizing 4 different separation technologies. Furthermore, we investigated a possible relationship between the steroid hormone concentration and the different manure fractions and separation technologies used. The chemical and steroid hormone analysis was done by inverse and integrated clean-up pressurized liquid extraction, and further cleaned by a two step solid-phase extraction before derivatization and finally analyzed by GC-MS/MS.

It was found that the steroid hormones were predominant in the solid manure separate calling for manure management strategies to reduce the content of steroid hormones in separated manure solid fraction. This could potentially be achieved through composting or anaerobic biogas production of the solid fraction; however, the effects of these technologies on steroid hormones need to be verified.

MO 192
Criteria for endocrine disruptors: report from the Danish centre on Endocrine Disrupters (CEHOS)
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The aim of this session is to give a presentation of the report (both ENV and HH) on criteria carried out by the Danish Centre on Endocrine Disrupters (CEHOS) as a project contracted by the Danish Environmental Protection Agency. CEHOS is an interdisciplinary scientific network without walls and the main purpose of the Centre is to build and gather new knowledge on endocrine disrupters (EDs) with focus on information needed for the preventive work of the regulatory authorities. The aim of the report was to propose scientific criteria for the identification of ED substances of concern for human health and the environment. A number of issues relevant for the development of criteria for EDs as well as data on the preliminary state of the art and relevant for the future work on endocrine disrupters were discussed.

The proposed criteria divide substances into three categories dependent on the available data: 1. Confirmed ED, 2a. Suspected ED and 2b. Indicated ED. The report describes the scientific evidence needed for fulfilling these criteria based on the OECD Conceptual Framework for endocrine testing and assessment. It considers non-test methods, test methods, epidemiological and field studies and gives examples of available ED data and relevant placement in groups. The overall purpose of the report is to provide scientific background for Danish input to the ongoing EU work within this field.

EP05P - Non-target analysis and identification of toxicologically significant emerging pollutants
MO 193
Innovative biodiagnosis meets chemical structure elucidation - New tools in effect directed analysis to support the identification and monitoring of emerging toxicaants on a European scale (EDA-EMERGE)
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Innovative biodiagnosis meets chemical structure elucidation - New tools in effect directed analysis to support the identification and monitoring of emerging toxicaants on a European scale (EDA-EMERGE)
Ionophores are the most heavily applied sub-group of the two sub-groups of anticoccidial agents, other hosts. The prevention and treatment of the disease coccidiosis, which is caused by a unicellular intestinal parasite. Coccidiosis is a major serious disease in poultry as well as in many other species.

Ionization pitfalls in nontarget screening by LC-high resolution mass spectrometry - Observation and application of normalised retention time factors reflecting the molecular hydrophobicity as identification criterion and allowing independent their likely degradation products. This results from (i) adduct formation with solvent or trace contaminant ions, (ii) gas-phase chemical reactions with solvents or trace contaminants, or (iii) electrochemically induced redox reactions at the electrospray needle. While available software tools can account for adducts with well-known species (Na+, NH4+ in positive ion mode; formic acid in negative ion mode) more complex substitution or redox reactions can hardly be addressed. We will present several case studies involving aromatic amines, nitroaromatic compounds or highly chlorinated weak gas-phase acids or bases. We will discuss approaches to allow for a correct assignment of their molecular formula in nontarget screening approaches and how the ions which are likely formed can be derived for different compound classes in suspect screening approaches.

MO 195
Ionization pitfalls in nontarget screening by LC-high resolution mass spectrometry
M. Kraus, C.D. Hug, W. Reck
UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany

In nontarget screening approaches using high-resolution mass spectrometry, one important step is the determination of the molecular formula from accurate measured mass and isotopic pattern. Similarly, the suspect screening approaches derive the exact mass and isotopic pattern from the molecular formula of the compounds of interest. In both cases the formation of protonated (positive ion mode) and deprotonated molecules (negative ion mode), is commonly anticipated when using electrospray (ESI) and atmospheric pressure chemical ionization (APCI). However, for a range of different compound classes, (de-)protonated molecules might be a minor species failing to reach selected intensity thresholds or even be completely absent. This results from (i) adduct formation with solvent or trace contaminants, (ii) gas-phase chemical reactions with solvents or trace contaminants, or (iii) electrochemically induced redox reactions at the electrospray needle. While available software tools can account for adducts with well-known species (Na+, NH4+ in positive ion mode; formic acid in negative ion mode) more complex substitution or redox reactions can hardly be addressed. We will present several case studies involving aromatic amines, nitroaromatic compounds or highly chlorinated weak gas-phase acids or bases. We will discuss approaches to allow for a correct assignment of their molecular formula in nontarget screening approaches and how the ions which are likely formed can be derived for different compound classes in suspect screening approaches.

MO 196
RISK-IDENT: assessment of previously unknown anthropogenic trace contaminants
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2Landeswasserversorgung Stuttgart, Stuttgart, Germany
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4Hochschule Weihenstephan-Triesdorf, Freising, Germany
5Technische Universität München, Freising, Germany

For the purposes of precautionary environmental protection, the project “RISK-IDENT” develops and applies an identification system for so far unknown anthropogenic trace contaminants in the aquatic system. The degradation of selected trace compounds is tested in lab-scale sewage treatment plants. Column and lysimeter studies are used to characterise mobility. Acute and chronic effect tests will contribute to the risk assessment process. Moreover an innovative elimination method as an additional sewage treatment step based on hydroxyl radicals will be proved. The starting point of the project is the development of a database for relevant aquatic contaminants which allows an attribution to analytical data from the analysis of real water samples. This is done by:

- Acquisition of substance data on the basis of REACH dossiers with particular reference to molecule-specific information (exact mass, log Pow, etc.) of these substances and their likely degradation products
- Observation and application of normalised retention time factors reflecting the molecular hydrophobicity as identification criterion and allowing independent interlaboratory HPLC methods
- Application of the three different LC-MS and LC-MS/MS techniques using high-accuracy mass spectrometry for detection of contaminants via the exact mass and multiple-reaction-monitoring (MRM) for target- and nontarget-screening strategies with low- and high-resolution tandem mass spectrometers
- Optimisation of an procedure for identifying previously unknown trace compounds through the computer-aided comparison of experimental features with molecule-specific properties of potential water contaminants

The project presents the recently launched project “RISK-IDENT” and illustrates first results of the interlaboratory determination of normalised retention time factors and mass spectrometric information on the way to an independent data base applicable for all laboratories working on the water sector.

“RISK-IDENT” is supported by the German Federal Ministry of Environment and Research (FKZ: 02WS1273A).

MO 197
Detection and fate of ionophores in the environment
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Anticoccidial agents or coccidiostatics are the only anti-bacterial substances still authorised as feed additives within the European Union. Anticoccidial agents are used for the prevention and treatment of the disease coccidiosis, which is caused by a unicellular intestinal parasite. Coccidiosis is a major serious disease in poultry as well as in many other species.

 Ionophores are the most heavily applied sub-group of the two sub-groups of anticoccidial agents, because they also have antibacterial properties. After the ban of antibiotic growth promoters ionophores are used extensively worldwide as prophylactic chemotherapeutics and growth promoters in livestock production. As an example, the yearly consumptions of active compounds are more than 10 tonnes in Denmark and for the Republic of Korea more than 800 tonnes. In long term, this may cause problems with resistance development in the treatment of coccidiosis.

Several reports have revealed that ionophores are emerging environmental contaminants in agricultural run-off waters, surface waters, sediments, and ground waters, due to their continuously increased and constant application as feed additives in modern livestock production. Recent investigations have further reported that transformation...
products of certain veterinary drugs such as antibacterial agents (i.e. tetracyclines) possess environmental effects on the soil-bacterial community at similar level as their parent compounds. This has previously also been observed for antibacterial agents.1

The focus of this study is on general analytical methods for detection of ionophores and unknown transformation products in various matrices. The hyphenated method consists of an integrated clean up with solid phase extraction followed by high-performance liquid chromatography tandem mass spectrometry (XPE-HPLC-MS-MS).

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MO 198
Prioritization of emerging pollutants on the basis of chemical structure

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The identification of unknown substances in complex environmental mixtures plays an important role in effect-directed analysis (EDA). Our approach for the identification of unknowns is based on the generation of possible structures followed by the progressive exclusion of structures that do not match experimental chromatographic and spectroscopic data simultaneously. Liquid Chromatography Elution Relationship (LSER) is applied as classifiers to predict the logarithmic retention factors log k in isocratic Gas Chromatographic (GC) measurements from the structures of candidate compounds. To demonstrate retention prediction and the application of the classifier model, twelve compounds with the molecular formula C_{12}H_{10}O_{2} were selected, while experimental log k values were compared to the predicted values and exclusion of potential candidate compounds was performed. Predicted retention factors gained from calculated structure descriptors show poor quality. Prediction was enhanced by using experimental determined descriptors, also achieved by the GC measurements.

MO 200
Linear solvation energy relationship models applied as classifiers in non-target analysis - a gas chromatographic approach

W. Brack, N. Ulrich

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The identification of unknown substances in complex environmental mixtures plays an important role in effect-directed analysis (EDA). Our approach for the identification of unknowns is based on the generation of possible structures followed by the progressive exclusion of structures that do not match experimental chromatographic and spectroscopic data simultaneously. Linear Chromatography Elution Relationship (LSER) is applied as classifiers to predict the logarithmic retention factors log k in isocratic Gas Chromatographic (GC) measurements from the structures of candidate compounds. To demonstrate retention prediction and the application of the classifier model, twelve compounds with the molecular formula C_{12}H_{10}O_{2} were selected, while experimental log k values were compared to the predicted values and exclusion of potential candidate compounds was performed. Predicted retention factors gained from calculated structure descriptors show poor quality. Prediction was enhanced by using experimental determined descriptors, also achieved by the GC measurements.

MO 201
Quantitative determination of different ether species in surface waters by Solid Phase Extraction and GC/MS

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Numerous ether species are of growing concern to human health as well as the environment. The production and use of ethers has been rising in many industrial sectors together with the exposure of these compounds to humans. There is a common lack of available information on the use, exposure and toxicity of these compounds. In this study a method was developed for the extraction and enrichment of six compounds from the ether family: 1,4-dioxane, dimethoxyethane, dimethyl ether and trimethyl ether. These compounds are of concern because they easily dissolve in water and do not partition to soils, entering ground water systems and likely contaminating public water supplies. They do not breakdown easily and might be difficult to remove from ground water sources. These compounds are also commonly used as reaction solvents in the area of pharmaceuticals or specialty chemicals production. Glycerol is a naturally occurring ether and has been found to be both toxic to the reproductive and development of a variety of hazards such as their parent compounds. We have developed methods for this analysis based on solid-phase extraction. The goals are to elucidate the environmental significance of this abiotic transformation and gain evidence to support a proposed mechanism.

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MO 202
UPLC/TOF/MS and in silico screening for transformation products of pharmaceuticals in water/sediment tests

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Pharmaceuticals are widespread pollutants in the aquatic environment. Laboratory studies showed that the microbial community in river sediments is capable of transforming a variety of pharmaceuticals, and in some river systems this process is the dominating attenuation mechanism. In order to discriminate attenuation of pharmaceuticals along a river from dilution, suitable tools are needed. Among others, the determination of characteristic transformation products could be one such tool. The aim of this study was therefore to elucidate the transformation of several pharmaceuticals in river sediment to evaluate this approach.
We carried out a series of incubations with sediment from three different rivers and synthetic river water. Tests were carried out at an initial concentration of 100-200 μg L⁻¹ for approx. 30 days; samples were filtered and subsequently analyzed by UPLC-qTOF-MS. The high-resolution mass spectrometric data were processed with a workflow combining the tools m/zMine (http://mzmine.sourceforge.net/) and envMass (http://www.eawag.ch/forschung/uchem/software/envMass)). By comparing incubations containing mixed samples to control samples, we determined the number of peaks identified by exact mass and retention time for each time step of the incubation experiment. Using these time series data, peaks with a temporal trend expected for transformation products (e.g., not present at the beginning of the incubation, increasing concentrations with time) can be located and processed further. Through application of this time series approach, the number of peaks to be processed further was substantially reduced.

The first results obtained by this approach are promising and provide a solid basis for the evaluation of the workflow, but also for scrutinizing its precision and robustness in detecting unknown peaks at low concentrations. This is necessary to increase confidence in the applied analytical and in silico methods for the specific aims of our study. We will present results of this evaluation, and we will discuss the tentatively identified transformation products with respect to their formation/elimination kinetics and their applicability as indicators for in situ micropollutant transformation.

MO 203 Evaluation of the contamination of aquatic micro-organisms by micro-QeUEChERS nano-LC-MS/MS

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It is currently accepted that the main route for pharmaceuticals and Endocrine Disruptors Chemicals (EDCs) to the aquatic environment is via sewage treatment plants receiving wastewater from households, industries and hospitals. In a follow-up to their findings and their impacts, we developed analytical methods to quantify endogenous and synthetic hormones, pharmaceuticals and chemicals (antibiotics) establishing a database in accordance with prioritisation lists [1] and identification of contamination sources around the wastewater treatment plant (industries, hospital, villages): estrene, 17α-ethinyl-estradiol, lорezapam, oxazepam, acetaminophen, carbamazepine, ibuprofen, 4-methylbenzylidene camphor, fluoroquinolones and sodium for example.

We decided to evaluate the contamination of three different species which are well-known bio-indicators of water quality. More precisely, invertebrates like gastropods (Potamopyrgus antipodarum), amphipods (Gammarus) and chironomidae larvae (bloodworms) are exposed to effluents from treatment plants.

In conclusion, the comprehensive method was applied to the study of the fate and bio-accumulation of preoccupying micro-pollutants in aquatic micro-organisms.

MO 204 Occurrence and seasonal variations of preservatives and UV filters used in cosmetic products in Japanese rivers

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Preservatives and UV filters are widely used in many cosmetic products. These compounds may enter the aquatic environment from showering, wash-off, and so forth via wastewater treatment plants or sewage treatment plant. Most of these preservatives are included in order to suppress microorganisms growing. UV filters are used to prevent skin damage from ultraviolet light. Some of them are hormonally active to aquatic organisms. Their ecological adverse effect on aquatic organisms should be concerned after discharge. But there is little published data of their occurrence in aquatic environment.

In this study, we determined twelve preservatives (2-Phenoxyethanol, Resorcinol, Chloroxylenol, 4-Isopropyl-3-methylphenol, Chlorphenesin, Triclosan, Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben) and four benzenophene-type UV filters (BP-1, BP-2, BP-3, BP-6) using solid phase extraction and GC/MS. Surface water samples were collected monthly at six sites in four Japanese rivers from December 2010. 2-Phenoxyethanol, Resorcinol, 4-Isopropyl-3-methylphenol and Triclosan were found in most of the samples in all seasons. In 2-Phenoxyethanol, Triclosan, BP-2 and BP-6 were not detected at sampling sites. The highest concentration was found for 2-Phenoxyethanol at 1 μg/L level. Further investigation is needed to evaluate the ecological effect of high concentration preservatives including 2-Phenoxyethanol.

MO 205 Solid phase extraction and LC-MS/MS analysis of pharmaceuticals in the Irish aquatic environment

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Pharmaceutical and personal care products (PPCPs) are resistant water remediation techniques carried out currently in wastewater treatment plants and are thus being released into receiving waters. In previous studies PPCP presence has been detected in Irish effluent and soil in measurable quantities. In the work presented herein, 24 hour composite effluent samples were collected from two large Irish wastewater treatment plants and analysed for a total of six pharmaceutical classes including non-steroidal anti-inflammatory agents, antibiotic, lipid regulators and anti-epileptics. The complexity of these sample matrices requires a clean up and extraction step and a multi-class instrumental analysis. For this study an innovative nano-LC coupled to tandem mass spectrometer. After cryo-grinding, we extracted samples with a salting-out assisted LLE followed by a purifying dSPE. The extract was evaporated then reconstituted in a mobile phase to be used in the LC analysis. A 45 minute mobile phase gradient of 20% acetonitrile with 13 mM ammonium acetate, pH6.2, to 80% acetonitrile) with UV detection followed by MS/MS detection. This is necessary to increase confidence in the applied analytical and in silico methods for the specific aims of our study. We will present results of this evaluation, and we will discuss the tentatively identified transformation products with respect to their formation/elimination kinetics and their applicability as indicators for in situ micropollutant transformation.
In this study 73 surface sediment samples were collected in China and investigated to the level, distribution, possible sources and different profile patterns of flame retardants (FRs) in the two countries. 25 German surface sediment samples were taken in the German Wadden Sea, the rivers Elbe and Weser and the German Bight in 2011. 48 Chinese samples were collected in Liaozhou Bay and discharging rivers in 2010. The samples were Soxhlet extracted with dichloromethane and then purified by 10% water deactivated silica before measured by GC-ECDN-MS. Quantification of 27 different FRs was obtained using 5 mass labelled internal standards [13C4 HHB, 13C6-BDE-77, 13C6-BDE-138, 13C6syn-DP, 13C6-BDE-209]. The concentration of DBCD in Liaozhou Bay was Decabromodiphenyl ethane (DBDPE) and BDE-209 in German Bight, respectively. The alternate BFR DBBPE showed higher concentrations than BDE-209 in most Liaozhou Bay except some Chinese river samples. Dibromopropyltribromophenyl ether (DPTF), Dichlorobenzene (Dec) 602 and 603 were found in German samples. Generally the concentrations of FRs in German Bight and discharging rivers were quite comparable, while the Chinese samples showed big differences between marine and riverine sediment contamination.

**MO 208**

**Water quality in the Three-Gorges Reservoir (China) - First results from the Yangtze Project WATERUSE**  
Institute for Environmental Studies (IVM) - VU Amsterdam, Amsterdam, Nederland

Among the key contaminants in the Yangtze river water, emerging pollutants (industrial chemicals, pesticides, pharmaceuticals, x-ray contrast media) and organic emerging pollutants (industrial chemicals, pesticides, pharmaceuticals, x-ray contrast media) are of particular interest. Databases and surveys have been made on potential sources (industrial facilities, medical centers, emergency hospitals) and the types of emerging pollutants. Effluent samples and natural water samples were collected from the Yangtze River and its tributaries in order to assess its suitability as drinking and irrigation water. In this paper, we will present the results of water quality monitoring in the Yangtze River and its tributaries.

**MO 209**

**EDA of extracts from two different passive sampling devices used in a polluted river network**  
A. Grun, C. Harman, I.J. Allan, K. Langford, S. Ranneklev, M. Muusse, K.V. Thomas  
Norwegian University of Life Sciences, Ås, Norway

In this study, two different types of passive sampling devices (silicone and LDPE) were deployed in the Alna River, a Norwegian river that has historically received pollution from a wide range of different industrial sources. The results of the study show that silicone seems to sample more of each compound, and a broader range of compounds, both in the relatively non-polluted area and from discharging rivers in 2009.

**MO 210**

**Target and non-target screening of lake sediments using HR-MS/MS**  
A.C. Chiaia-Hernandez, H. Stinger, J. Hollender  
Eawag, Dübendorf, Switzerland

Non-target screening of lake sediments was performed using high resolution mass spectrometry (HR-MS/MS) to obtain information on the occurrence and distribution of priority pollutants in lake sediments. The results show that HR-MS/MS is a powerful tool for the detection and quantification of priority pollutants in lake sediments.

**MO 211**

**Characterization of surface water by multi-target screening of organic micropollutants combined with toxicological assessment**  
Fraunhofer Institute for Environmental Chemistry, Jena, Germany, and University of Leipzig, Leipzig, Germany

In the past, several environmental and toxicological studies focused on persistent organic pollutants in domestic and industrial waste water. Due to incomplete retention in water treatment plants (WTPs), toxic chemicals are yearly discharged to surface water. Although occurring in low concentration, these micropollutants might harm aquatic organisms. Especially mutagenic and endocrine disrupters are of great concern due to their potential effects on ecosystems. The aim of this study was to link the complex chemical composition of surface water samples with their multifactorial effects on organisms. Therefore, surface waters downstream of WWTPs associated to different sources (domestic, agricultural, industrial waste water) were sampled. The samples were analysed by liquid chromatography-high resolution mass spectrometry for almost 300 environmental relevant substances, such as pharmaceuticals, pesticides, industrial chemicals or ingredients of personal care products. For biological characterization, the samples were tested for mutagenic and estrogenic effects by an Ames-test II and a zebrafish egg test, respectively. Obtained data were evaluated by chemometric tools such as partial least-squares regression. Chemical fingerprints and toxicological endpoints were correlated by a multivariate regression model. Groups of chemicals causing similar effects on organisms, as well as possible marker substances for waste water sources will be presented.

**MO 212**

**Known unknowns: strategies for identification of toxicants in Effect Directed Analysis**  
Institute for Environmental Studies (IVM) - VU Amsterdam, Amsterdam, Nederland

Toxicological characterization in the field of analytical chemistry has contributed significantly to the potential to find, identify and quantify unknown compounds present in the environment. Potentially, the application of accurate mass spectrometric techniques (e.g. time-of-flight, Orbitrap, FTMS) in environmental analysis can facilitate the
identification of unknown toxicants. One of the research areas that would benefit enormously from improvements regarding the identification of compounds is the field of Effect Directed Analysis (EDA). Our current work is dedicated to the development of a generic workflow aimed at the identification of compounds in extracts that are of potential interest. In our research, we have calculated both emission data on loads to identify these mixtures and used available chemical analytical methods to identify known substances. However, the capacity to measure these emerging compounds is yet limited. For that reason, we have adopted a new bioassay that can be included as screening tool for these compounds. In our research, we have calculated both emission data on loads to identify these mixtures and used available chemical analytical methods to identify known substances. However, the capacity to measure these emerging compounds is yet limited. For that reason, we have adopted a new bioassay that can be included as screening tool for these compounds.

Rapid acute toxicity screening of commercial and potential green plasticizers using bioluminescent bacteria

MO 218

The Vibrio fischeri bioluminescence inhibition assay (Microtox®) using an in-house diluent containing 2% DMSO was used to perform a rapid toxicity screening of 24 commercial and potential green plasticizers. This bioassay allowed us to better understand the impact of a key structural feature, alkyl chain size, on the toxicity of the target plasticizers. Results indicated that dibenzoate compounds with lower number of C atoms in their alkyl chains, such as 1,3-propanediol dibenzoate (PrDDB) and 1,4-butanediol dibenzoate (BDDB), were more toxic (EC₅₀ = 2-6 µM) than the commercial plasticizers di(2-ethylhexyl) phthalate (DEHP), 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH) and di(2-ethyl hexyl) adipate (DEHA), which did not inhibit the bacterial luminescence. Nevertheless, diethyl and di(2-ethylhexyl) phthalate compounds are weakly toxic (EC₅₀ = 130-1292 µM) or non toxic at all to the luminescent bacteria. These data suggest that the concentrations of sucralose detected in the environment are well below those required to elicit chronic effects in freshwater or marine invertebrates. Data will also be presented with respect to findings in the fish ELS study.

MO 215

S. A. E. Kools

Sucralose is an intensively sweet food additive derived from sucrose in a process where three hydroxyl groups are substituted for three chlorine atoms, producing a very stable substance and has become a popular substitute for sugar. Its exceptional stability in combination with high water solubility and popularity has resulted in measurable concentrations not only in recipient waters but also further out in the oceans. Animal and human studies have concluded that sucralose is safe for human use and earlier studies in aquatic organisms indicate low bioaccumulation potential and negligible acute/chronic toxicity. The close structural resemblance with sucrose in combination with the importance of sugar in nature implies that other assessment techniques than traditional methods might be of interest. The aim of this investigation was therefore to combine traditional methods with behavioral studies in crustaceans and algae. Our research indicates that sucralose is not statistically significant reduction in D. magna survival or reproduction. Survival, growth and reproduction of mysid shrimp were unaffected by ≤ 93 mg/L sucralose. Collectively, these data suggest that the concentrations of sucralose detected in the environment are well below those required to elicit chronic effects in freshwater or marine invertebrates. Data will also be presented with respect to findings in the fish ELS study.
The purpose of this study was to evaluate the exposure to arsenic (As), barium (Ba), cadmium (Cd), mercury (Hg) and lead (Pb) in three raptor species from different areas of Portugal. Birds of this study were brought alive, but injured or debilitated for various reasons, to four Portuguese Wildlife Rehabilitation Centers. Blood samples were collected from 72 common buzzards (Buteo buteo), Goshawks (Accipiter gentilis), Short-toed Eagle (Circaetus gallicus) and Kentish Plover (Charadrius alexandrinus) from the Tagus Estuary, Portugal.

The ingestion of Pb shot used for hunting constitutes the main cause of lead poisoning in waterfowl. Females may transfer some of the accumulated Pb to their eggs and, although most Pb is expected to accumulate in the eggshell, it can ultimately affect embryonic and hatching health and development. We collected eggs of mallard ducks from the Ebro delta (NE Spain), an area with high Pb shot prevalences in waterfowl, and incubated them artificially. Blood samples were collected at days 3 and 28 post hatching in mallard ducklings (b-ALAD activity, oxidative stress indicators, biochemical parameters, vitamins and carotenoids). Cellular immune function was quantified at day 14 as the intensity of swelling produced in response to an intradermal injection of phytohemagglutinin (PHA). Humoral immune function was measured at day 28 as the amount of antibodies synthesized against an intraperitoneal injection of sheep erythrocytes. Detections with Pb blood levels above 180 ng/ml died during the first 7 days post hatching. Eggshell Pb levels were significantly correlated with blood Pb levels in ducklings at day 3 (r=0.549, p<0.001), but not at day 28. b-ALAD activity and cellular immune function were negatively correlated with Pb levels in eggshell (b-ALAD : r=-0.383, p=0.040; PHA reaction: r=-0.471, p=0.048) and blood of mallard ducklings (b-ALAD : r=-0.446, p=0.009; PHA reaction: r=-0.639, p=0.003). Neither the humoral immune function nor the rest of blood parameters were affected by blood or eggshell Pb content.

Blood concentrations of heavy metals (Ba, Cd, Hg and Pb) and Metalloid (As) in raptor species (Buteo buteo, Milvus migrans and Gyps fulvus) from Portugal estimate the effects of Pb intoxications.

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Blood concentrations of heavy metals (Ba, Cd, Hg and Pb) and Metalloid (As) in raptor species (Buteo buteo, Milvus migrans and Gyps fulvus) from Portugal estimate the effects of Pb intoxications.
and reproduction tests were conducted with earthworms. Cocoon production was lower in shotfall soil compared to reference soil and Webster (laboratory standard soil).

**MO 245**

**Effects of lead shot ingestion on constitutive immune function of red-legged partridges**

N. Valverde, M.E. Ortiz-Santalesta, J.M. Pérez de la Lastra, R. Mateo

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The use of agrochemicals is suspected to be a major cause of population declines of farmland birds from Western Europe. Apart from indirect effects like reduction of available food and optimal habitat, ingestion of pesticide-coated seeds constitutes a potential way of direct intoxication. Although the most toxic chemicals are being legally restricted, some of the pesticides currently used for seed coating can cause adverse effects when consumed by birds. The aim of the present project is to test the effects of coated seed ingestion on red-legged partridge (Alectoris rufa) physiology, fitness and reproduction. We tested two insecticides (natural pyrethrin and imidacloprid) and two fungicides (mancozeb and thiram). Each experiment consisted in a group of 16 pairs of partridges exposed to two doses: a high dose corresponding to recommended for seed coating and a low dose being the 20% of the recommended one. Exposure was split in two periods, corresponding to the two seasons of cereal sowing: 25 days in autumn and 10 days in late winter. Imidaclopirid was the most toxic compound, killing all partridges at the recommended dose in 21 days. All pesticides affected hematocrit (controls: 42.3%; treated: 38.3-41.0%), whereas mancozeb at high dose reduced body condition after the first exposure period (10.6% lower than controls). Reproduction was affected especially by the fungicides, and at low doses rather than at high ones. The two fungicides reduced the fecundation rate (34.54% vs. 62% in controls) and thiram caused a significant decrease in chick body condition 24 days after hatching (18% lower than controls) that ultimately caused a marginally significant mortality (p=0.07). At high dose, this effect could real, but not at low dose. The most important characteristic in bone mineralization of Pb-exposed adult deer was a lower content in bone mineral that was associated with the increased free retinol percentage, which in turn had consequences on bone biomechanics. Although Pb effects on wild boar have been less significant, the degree of bone mineralization was also positively associated with higher burdens of retinyl esters. In conclusion, we suggest that changes in antioxidant vitamins found in deer may be involved in the toxic effects of Pb on bone composition.

**MO 246**

**Assessment of fungicide- and insecticide-coated seed risks for red-legged partridges**

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**MO 247**

**Long-term effects of Pb pollution on mineralization and biomechanical properties of bone in wild ungulates: relationship with vitamins A, D and E**


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Departamento de Geología, Facultad de Geología, Universidad de Oviedo, Oviedo, Spain

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The effects of toxic levels of Pb are diverse and represent several functional systemic alterations. Although the effects of exposure to Pb on the immune system of mammals, and especially in humans, have been extensively studied, little is known about how this heavy metal affects the constitutive immune function in birds. Pb intoxication may be a major problem for birds as they are contaminated with grt particles. We present a preliminary study based on different tests to assess changes in innate immune response of red-legged partridges (Alectoris rufa) subjected to Pb shots. We used four experimental groups (0-9 Pb shots) with six replicates each. A marginally significant lethal effect was detected for the highest Pb treatment (60% mortality; p=0.057). Among survivors, decrease in body mass after Pb exposure affected at 3.5 days after exposure. We compared blood Pb levels, analyzed by Atomic Absorption Spectrophotometry with graphite furnace, with different parameters indicative of the innate immune response. The ratio between lymphocytes expressing CD4 and CD8 membrane receptors, analyzed by flow cytometry, was affected by the interaction between treatment and sex in a less than significant manner (p=0.060). Haematocrit, serum lysozyme and myeloperoxidase activities were affected by the exposure to Pb. Our results highlight the necessity to analyze several parameters to assess the immunotoxicity of a substance. Nonetheless, other tests should be repeated with a larger sample size to obtain meaningful results. Analyzing constitutive immune function is important in wild populations as it represents the organism’s ability to display a quick response against opportunistic pathogens.

**MO 248**

**ET09P - Global climate change: implications for environmental toxicology and chemistry**

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**MO 249**

**The influence of global climate change on the scientific foundations and applications of environmental toxicology and chemistry: summary from a SETAC international workshop (July 2011)**

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This is an introduction to and summary from a SETAC international ‘Pellston’ workshop titled “The Influence of Global Climate Change on the Scientific Foundations and Applications of Environmental Toxicology and Chemistry”. Held July 16-21, 2011 it involved some 36 scientists from 11 countries. The central question addressed was: How will global climate change influence the environmental impacts of chemicals and other stressors; and, the way we assess and manage chemical contamination and other stressors in the environment? While more detail is found in the complete series of papers, some key consensus points are: 1, human actions (adaptation, mitigation, etc.) may have much or more influence on the fate and effects of chemical contaminants as does GCC, and modeled predictions should be interpreted cautiously; 2, effects of GCC may be slow, variable and difficult to detect though some species and populations of high vulnerability (polar, coral reefs) may exhibit responses sooner and more dramatically than others; 3, responses to GCC may lead to new environmental issues while at the same time, traditional chemical risk assessments will need to incorporate multiple stressors, cumulative risks (positive and negative) considering the wide spectrum of potential impacts stemming from GCC; 4, field / reference conditions for estimating resource injury and restoration / rehabilitation will continually shift due to GCC and represent significant challenges to practitioners.

**MO 250**

**Consequences of metal adaptation on sensitivity to future climate change stressors in Daphnia pulex populations**

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Genetic adaptation may allow for natural populations to adapt to a stressor, which is manifested by an increased tolerance to the stressor. As a consequence of this genetic adaptation, populations may also become more (co-tolerance) or less tolerant (cost-of-tolerance) to other stressors. In this research we investigated the cost-of-tolerance hypothesis in the context of climate change, i.e. that populations of the waterflea Daphnia pulex that adapted to cadmium (i.e. more tolerant to sublethal cadmium concentrations) are less tolerant to exposure to other climate change stressors, cyanobacteria and hypoxia. A common stressor to most zooplankton species and are predicted to play an increasingly prominent role in aquatic ecosystems as their occurrence in harmful algal blooms is predicted to increase due to climate change. Increasing deposition of atmospheric nitrogenous gases and phosphorus loading from agricultural fertilization and fossil-fuel emissions into the world’s surface waters will substantially increase the influence of these waterbodies, eventually resulting in an increased amount of sinking organic matter. Hypoxia is caused by an increase in microbial oxygen requiring processes which cope with digestion of this organic matter. Furthermore, higher water temperatures tend to increase the duration and strength of thermal stratification by reducing the mixing of oxygen rich water layers with oxygen poor layers, resulting in a prolonged hypoxia zone. To test the cost-of-tolerance hypothesis, animal, were exposed to either a diet containing 50% Microcystis aeruginosa, 2 mg.L-1, O2, or 0.5 µg Cd.L-1 to assess cyanobacteria, hypoxia, and cadmium tolerance, respectively. Control animals were maintained at an oxygen level of 9 mg.L-1 O2, and were fed 100% Anakdosostrea falcatus. Tolerance was assessed as control normalized.
reproduction and growth. Contrary to the cost-of-tolerance hypothesis, we found significant correlations between cadmium and both M. aeruginosa and hypoxia tolerance, suggesting that co-tolerance rather than a cost-of-tolerance occurred. These findings suggest that cadmium adapted Daphnia populations are better suited to face some future climate change stressors than non-cadmium adapted populations.

MO 251  
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CIMAR - Instituto do Mar, Department of Life Sciences, University of Coimbra, Coimbra, Portugal  

Health condition of Minho and Lima Corbicula fluminea populations in relation to summer mass mortality events.  
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CIIMAR, Porto, Portugal  

MO 254  
Temperature-dependent effects of the fungicide pyrimethanil on the reproduction of Physella acuta  
A.S. Seeland, R. Muller, J. Oelmann  
Goethe University, Frankfurt, Germany  

In vitro testing of biofuels: Verifying the environmental friendliness of biofuels in comparison with fossil fuels.  
S. Heges', K. Blueml', T.B. Seiler', A. Schaffer', H. Hollert  
Institute for Environimental Research, RWTH Aachen University, Aachen, Germany  

MO 252  
MO 255  
In vitro testing of biofuels - Verifying the environmental friendliness of biofuels in comparison with fossil fuels  
S. Heges', K. Blumhl', T.B. Seiler', A. Schaffer', H. Hollert  
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To understand the sublethal impact of contaminants and how temperature influences sensitivity of molluscs as non-model organisms we assessed an embryo toxicity test as a combination of multiple stressors may lead to severe alterations for organisms such as changes in time of reproduction, reproductive success and growth performances, mortality and geographic distribution. Recent research revealed that aquatic model organisms tend to react more sensitive to chemical stress effects. To go further on the causes contributing to such die-offs, a monitoring study investigating the health status of the population in relation to water quality and other abiotic variables has been carried out. Using additive modelling, we found that 76% and 50% of the variability in evenness and richness can be explained by lake identity and the degree of land disturbance. Addition of more predictors (including Cu concentrations) did not improve model fit. However, the fitted lake-specific effects indicated that the two lakes located next to metal smelters (Cu up to 3000 µg/g) had considerably higher copper concentrations (up to 40 µg/g) than the other two lakes (Cu up to 500 µg/g). The beneficial effects of such die-offs are better understood when the铙。
This study was carried out in the scope of the project “NISTRACKS - Processes influencing the invasive behaviour of the non indigenous species Corbicula fluminea (Mollusca: Bivalvia) in estuaries - identification of genetic and environmental key factors” funded by the Portuguese Foundation for the Science and the Technology (FCT) (PTDC/AAC-AMB/102121/2008) and FEDER COMPETE funds (FCOMP-01-0124-FEDER-008556). C. Oliveira had a PhD grant from FCT (SFRH/BD/84230/2010) funded by national funds of the MCTES and FEDER European funds (POPH-QREN-Typeology 4.2.).

M.O. 256

Dichloropropene proteome responses of larvae of the marine medaka Oryzias melastigma upon cadmium exposure at thermal extremes

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Upon facing unpredictable extreme temperature events under the human-driven climate change, marine ectotherms would generally be more susceptible to additional stressors like toxicants. This study aimed to reveal the stress responses in marine medaka larvae Oryzias melastigma upon exposure to cadmium (Cd) under different thermal conditions including extreme temperatures. Larval fish were thermally treated by elevating or decreasing temperatures at a rate of 1°C per hour followed by 7 days of acclimation at each experimental temperature, before commencement of 4 doubling times of Cd treatments. The associated toxic stress responses were elucidated using two-dimensional gel electrophoresis (2-DE) coupled with multivariate statistical analyses. Proteins of fish larvae from temperature treatment groups (i.e., 10°C, 25°C and 32°C) were successfully separated using IEF strip with pH 3-10 and gradient SDS-PAGE with gel percentage of 4-12.5%. The total- and phospho-proteins were visualized using Sypro Ruby and Pro-Q Diamond stains, respectively. Similar numbers of total protein spots (from 206 to 225) were detected in the three-temperature treatment groups. Protein spots number 104, 48, 72 or 96nM, respectively depending on the temperature. Embryological analysis was carried out to determine percent developmental anomalies and/or embryonic mortality. The median effective concentrations (EC50) were 65,9±8,3nM at 17°C, 83,1±15,2nM at 20°C, 81,5±10,8nM at 23°C and 35,9±19,6nM at 26°C for S. granularis embryos. The results of this study could provide certain essential and fundamental information to further advance our understanding on specific toxic effects of trace metals under temperature extremes.

M.O. 257

The influence of fluctuating temperature on the effect of copper toxicity in Caenohabditis elegans

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Climatic changes in recent decades have increased the number and tempo of extreme events, which have been shown to affect the toxic effect of some chemicals. Temperature especially has a direct effect on the toxicity of metals and pesticides, generally causing toxicity to rise with increasing temperature, in particular when approaching the temperature tolerance limit of the test organism. Studies on the effect of temperature differences on toxicity have so far been done at static temperature regimes, which ignore any additional stress which may be caused by fluctuating temperature. Since daily temperature changes in the terrestrial environment can fluctuate with more than 10 degrees, it is questionable whether information from static regimes can be extrapolated to describe the effect of temperature on toxicity in the field. We hypothesize that fluctuating temperatures will prove to be a stress factor in itself, which may increase the effect of a toxicant, as compared to the equivalent static scenario at the average temperature. In the present study we expose the nematode Caenohabditis elegans to a range of copper concentrations at three static temperatures (11, 16 and 20°C), as well as in a non-static temperature scenario with a daily temperature range of ±1°C from 12.0 to 20.0°C and an average daily temperature of 16°C. The temperature exposures were chosen to be within the approximate optimal temperature range of C. elegans. The study use full life cycle exposures, with daily measurements of reproduction and survival during the entire lifespan. To our knowledge this is the first study to compare the effect of static and fluctuating temperature on toxicity of copper.

M.O. 258

Effect of temperature on embryotoxicity of the antifouling biocide zinc pyrithione to Sphecerechinus granularis

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The present study investigated combined effects of the antifouling biocide zinc pyrithione and different temperature (17, 23 and 26 °C, control at 20 °C) on embryonic development, hatching success and survival of Sphecerechinus granularis embryos. Simultaneous treatments of the toxicant-protein spots (from 206 to 225) were detected in the three-temperature treatment groups. Protein spots number 104, 48, 72, 96 and 99nM, respectively depending on the temperature. Embryological analysis was carried out to determine percent developmental anomalies and/or embryonic mortality. The median effective concentrations (EC50) were 65,9±8,3nM at 17°C, 83,1±15,2nM at 20°C, 81,5±10,8nM at 23°C and 35,9±19,6nM at 26°C for S. granularis embryos. Our results verified that toxicities of the zinc pyrithione, were highly temperature-dependent. The EC50 values of S. granularis exposed to zinc pyrithione decreased with increasing temperature between 20-26°C, whereas the EC50 values at 17°C were significantly lower than 20°C. To conclude, higher temperatures increased the sensitivity of S. granularis embryos to zinc pyrithione stress.

M.O. 259

Temperature-dependent toxicity of DDT to larvae of the marine medaka Oryzias melastigma: pollutant responses at thermal extremes

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In view of the progressive decrease in whole-organism aerobic scope and shift toward anaerobic metabolism under the extreme temperatures, we used extra cellular repairing processes under chemical exposure, adverse toxic responses could be expected under the extreme temperatures. Proteomic response of the fish larvae under combined Cd and thermal stresses is under investigation. The results of this study could provide certain essential and fundamental information to further advance our understanding on specific toxic effects of trace metals under temperature extremes.
The long-term use of Cu containing pesticides is assumed. It is understood that long-term accumulation of Cu in soils due to the annual application of limited amounts of Cu, as is the case for Cu containing plant protection products, may result in significant attenuation of Cu availability with time and hence a lower toxicity of Cu in soils compared to the default assumptions in the VRAR on the effect of ageing on toxicity. The effect of ageing is commonly taken into account in the effect assessment through the application of a so-called ageing-aging factor. Therefore, a larger scenario-specific ageing-aging factor is proposed for the effects assessment of Cu in soils affected by long-term application of Cu containing pesticides.

MO 263 Toxicity of Tributyltin (TBT) on terrestrial invertebrates and plants A.R. Silva, P.V. Silva, S. Mendo, S. Loureiro  
Department of Chemical & Environmental Sciences, University of Aveiro, Aveiro, Portugal  
University of Aveiro & CESAM, Aveiro, Portugal  

Tributyltin compounds are highly toxic organotins that were deliberately introduced into the marine environment by antifouling paints since 1960s. Their slow degradation rate and the TBT found in sediments and contributed to the contamination of TBT found in sediments. Their disposal on land as well as the effects of contaminated sewage sludge and application of TBT-based biocide products on agricultural fields constitute an important source of TBT contamination in soils. Due to their adverse effects to non-target organisms at very low concentrations (ng L-1) and bioaccumulative properties, TBTs are a hazard to soil flora and fauna. This study aimed to assess the effects of TBT on invertebrates and plants species. An agriculture soil collected from Portugal was used for the TBT exposure. For this evaluation several bioassays were performed: two feeding inhibition assays (with contaminated soil and with contaminated food) and one avoidance behaviour test were carried out with the model species Porcellionides pruinosus. Likewise, a reproduction test with the collombolan Folsomia candida and seed germination and plant growth test with the plant Brassica rapa and common wheat Triticum aestivum were also performed. Feeding parameters (consumption, assimilation and egestion ratios) and TBT survival decreased when exposed to TBT. P. pruinosus was able to detect very low concentrations of TBT in soil and thus could possibly escape from the contaminated sites. A dose related response between the decrease in the number of juvenile springtails and the increase in TBT concentrations in soil was also observed. For the plants' exposures a dose-response relationship was observed, showing a delay and decrease on seed germination in both plant species B. rapa and T. aestivum. With increasing tributyltin concentrations, a decrease on growth parameters, including biomass production and plants length, was also observed.

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Long-term effects of zinc oxide nanoparticles to soil (Glycyrrhiza max (L.) Merrill) grown were evaluated in the OECD standard soil. The OECD standard soil was amended with 10, 50, 150, 500 mg kg-1 of ZnO nanoparticles (< 50 nm). Plant experiments were conducted in a greenhouse. The growth and developmental stages of soybean were measured during exposure period of 8 to 9 weeks. The results showed that development of soybean was significantly affected at 500 mg kg-1 dry soil. This developmental delay led to a failure in the formation of seeds. The results demonstrated that ZnO nanoparticles affected negatively the life cycle of soybean.


Phosphogypsum (PG) is a metal and radionuclides-rich by-product of phosphoric acid production. For every tonne of phosphoric acid made, from the reaction of phosphate rock with acid, commonly sulfuric acid, about 3t of PG are created. There are three options for managing PG: (i) disposal or dumping (ii) stacking and (iii) re-use in agricultural use. Despite some authors have suggested the use of PG as a fertilizer, till our knowledge, till now any evaluation was made about the real impacts of soil amendment with PG, on soil fertility and reproduction functions. In this work reproduction assays with earthworms (Eisenia andrei), collombolans (Folsomia candida), enchytraeids (Enchytraeus crypticus) and mites (Hypoaspis aculeifer) and seed germination and growth assays with plants (Zea mays and Lactuca sativa) were performed to assess the toxicity of earthworms, parasites and the bacteria. In our study we wanted to examine the resilience of the former meadow community after its transfer into TME steel cylinders. We present data on the population dynamics of springtails, earthworms and the meadow and soil health, which contribute to a discussion on what extent TME can reflect natural conditions. For the plants' exposures a dose-response relationship was observed, showing a delay and decrease on seed germination in both plant species B. rapa and T. aestivum. In our study we wanted to examine the resilience of the former meadow community after its transfer into TME steel cylinders. We present data on the population dynamics of springtails, earthworms and the meadow and soil health, which contribute to a discussion on what extent TME can reflect natural conditions.

MO 266 Phosphogypsum as a soil fertilizer: evaluation of the impacts on soil habitat and retention functions I.O. Hentati,a L.A. Ana,b B.S. Bouguerra,a A.N. Abrantes,a G.F. Goncalves,b R.J. Rombe,c P.R. Pereira,c 1 High Institute of Biotechnology of Sfax, Sfax, Tunisia b Dep. of Biology, Fac. Ciências, Universidade do Porto & CESAM, Univ. Aveiro, Aveiro, Portugal c TCT Oekotoxikologie GmbH, Rottergrasse, Flörsheim, Germany, Flörsheim, Germany  

Phosphogypsum (PG) is a metal and radionuclides-rich by-product of phosphoric acid production. For every tonne of phosphoric acid made, from the reaction of phosphate rock with acid, commonly sulfuric acid, about 3t of PG are created. There are three options for managing PG: (i) disposal or dumping (ii) stacking and (iii) re-use in agricultural use. Despite some authors have suggested the use of PG as a fertilizer, till our knowledge, till now any evaluation was made about the real impacts of soil amendment with PG, on soil fertility and reproduction functions. In this work reproduction assays with earthworms (Eisenia andrei), collombolans (Folsomia candida), enchytraeids (Enchytraeus crypticus) and mites (Hypoaspis aculeifer) and seed germination and growth assays with plants (Zea mays and Lactuca sativa) were performed to assess the toxicity of earthworms, parasites and the bacteria. In our study we wanted to examine the resilience of the former meadow community after its transfer into TME steel cylinders. We present data on the population dynamics of springtails, earthworms and the meadow and soil health, which contribute to a discussion on what extent TME can reflect natural conditions.


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MO 268

Using the comet assay to determine species sensitivity of earthworms to copper
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The endpoints normally used to construct Species Sensitivity Distributions (SSDs), comparing the various sensitivities of species to toxicants, are predominately whole body or life-cycle characteristics. Biomarkers are easy and rapid tests. This study investigated whether a biomarker, the comet assay, could be used to compare species sensitivity between copper and different species. For this assay, measuring DNA damage in the form of single strand breaks, gives an indication of the genotoxicity of a toxic substance for an exposed organism. Four earthworm species from three different families were experimentally exposed to copper in the form of the fungicide, copper oxychloride. The species were used as lumbricid species, Eisenia andrei, two megascolecid species Perionyx excavatus and Amyntas diffringens and Chilopoda spp., an endemc South African species of the family Aculodeschild.

At toxic concentrations, Cu can be damaging on various levels and, as shown by the results of the present study, also acts as a genotoxic agent. Adult specimens were exposed to the fungicide in artificial OECD soil, with benomyl as positive control. An exposure concentration series of 0, 20, 80, 160, 320 and 640 mg/kg Cu was used. Exposures were done to juvenile worms for a duration of 14 days. After the exposure period, the worms were harvested from the organisms and the cells prepared for the comet assay according to the method of Singh et al. (1988) as adapted by Reinecke & Reinecke (2004).

The Tail DNA % parameter was used to determine the comet values. The highest mean damage to the DNA at all exposure concentrations was observed in A. diffringens and the least sensitive species Eisenia andrei, determined and SSDs constructed using EC10 and LOEC values. These showed that A. diffringens was the most sensitive and P. excavatus the least sensitive species of the four tested. We conclude that a biomarker, such as the comet assay, can give reliable and useable data from which to infer sensitivity differences between species.

MO 269

Determining chemical effects on earthworms at the population level through the use of energy budget and individual based modelling
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4Standard OECD test methods for measuring the toxicity of pesticides on earthworms use optimal laboratory conditions to record specific endpoints e.g. mortality, growth and reproduction. These data are compared to exposure values in accordance with EU guidelines for registration of agrochemicals, and the resulting risk quotients are compared to arbitrary safety factors for acute and sublethal risks to earthworms. When these triggers are not passed, field trials are expensive, time consuming and variable, providing coarse information about population-level effects. Mechanistic population modelling of organism responses to chemicals has the potential to act as a reliable intermediate stage in higher tier risk assessments and can provide greater resolution to help identify key parameters driving population-level effects.

Energy budget models relate individual life cycle processes to one another through energy and mass conservation. Organisms uptake resources from their environment and expend assimilated energy on maintenance, growth and reproduction. The allocation of energy to these processes depends on a combination of environmental and organism-specific conditions. Here we present a general energy budget model, based on broadly accepted fundamental principles. The model is applied within an individual-based model (IBM) framework, which allows individuals to interact with their simulated environment, giving an insight into life cycle processes affected under variable environmental conditions. This enable us to model overall population dynamics under realistic field conditions, as the biology of individuals relates to the ecology of populations through metabolism. Standard toxicity data, including NOEC and EC50 values, can be used as input data to the model, highlighting its usefulness as a method in higher tier risk assessments. We use data for the OECD recommended earthworm species Eisenia fetida to validate the model for use with real biological effects. We discuss the potential of the model in decision-making for pesticide regulation and environmental management, particularly in comparison to alternative modelling techniques. Future studies aim to utilize the flexibility of the model to extrapolate population-level ecotoxicological effects between the varied ecological niches of different earthworm species.

MO 270

Ecotoxicity of phenanthrene and its nitrogen-containing analogues to the earthworm Eisenia fetida in soil
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2Sustainable Development, University of Neuchatel, Switzerland
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Earthworms are used as test organisms in environmental pollution assessment. Used in studies of acute and chronic toxicity determining how response variables such as mortality, growth and reproduction change in response to changes in pollution levels. Earthworms are very sensitive and can respond to low concentrations and is sensitive to the overall toxic action and the relative degree of the toxic action, as illustrated by the significant ecotoxicological properties observed in the earthworm Eisenia fetida. The effects were studied over a 21-day incubation, during which health and mortality were assessed. During the 60 day incubation, LC50 and EC50 ranged from nd-606 mg/kg and 4.8-574 mg/kg respectively. NOEC levels were derived from EC10 values: estimated NOECs ranged from 0.01 to 204 mg/kg. LC50 values were estimated and compared to other published literature and our results differed significantly. The results of this study showed that NHAs were more toxic in soil than homocyclic PAHs (with aging). This can be attributed to their higher solubilities and lower Kow's. Based on visible pathological changes, NHAs were not only carcinogenic, mutagenic, teratogenic and genotoxic, they may also have been clastogenic to soil organisms especially benz(b)quinoine. These differences might be useful in soil contamination / risk assessment procedures and also provides basis for regulatory action.

MO 271

Toxicity of the earthworm Eisenia fetida of two transformation products ofamines used in carbon capture technology
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2Norwegian University of Life Sciences, As, Norway
3Norwegian Institute of Water Research, Oslo, Norway

The aim of this study was to assess the acute and chronic toxicity to the earthworm Eisenia fetida of two transformation products of amines, dimethylintraimine (DMIA) and 2-nitroamine (NAE) (N02C), using standard OECD guidelines. DMIA and NAE are nitrogen heterocyclic molecules (NHAs) which act as environmental contaminants. Previous research has shown that DMIA and NAE are toxic to the earthworm Eisenia fetida. The effects were studied over a 21-day incubation, during which health and mortality were assessed. During the 60 day incubation, LC50 and EC50 ranged from nd-606 mg/kg and 4.8-574 mg/kg respectively. NOEC levels were derived from EC10 values: estimated NOECs ranged from 0.01 to 204 mg/kg. LC50 values were estimated and compared to other published literature and our results differed significantly. The results of this study showed that NHAs were more toxic in soil than homocyclic PAHs (with aging). This can be attributed to their higher solubilities and lower Kow's. Based on visible pathological changes, NHAs were not only carcinogenic, mutagenic, teratogenic and genotoxic, they may also have been clastogenic to soil organisms especially benz(b)quinoline. These differences might be useful in soil contamination / risk assessment procedures and also provides basis for regulatory action.

MO 272

New earthworm endpoints based on coelomocytes
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Earthworms are used as test organisms in environmental pollution assessment. Used in studies of acute and chronic toxicity determining how response variables such as mortality, growth and reproduction change in response to changes in pollution levels. Earthworms are very sensitive and can respond to low concentrations and is sensitive to the overall toxic action and the relative degree of the toxic action, as illustrated by the significant ecotoxicological properties observed in the earthworm Eisenia fetida. The effects were studied over a 21-day incubation, during which health and mortality were assessed. During the 60 day incubation, LC50 and EC50 ranged from nd-606 mg/kg and 4.8-574 mg/kg respectively. NOEC levels were derived from EC10 values: estimated NOECs ranged from 0.01 to 204 mg/kg. LC50 values were estimated and compared to other published literature and our results differed significantly. The results of this study showed that NHAs were more toxic in soil than homocyclic PAHs (with aging). This can be attributed to their higher solubilities and lower Kow's. Based on visible pathological changes, NHAs were not only carcinogenic, mutagenic, teratogenic and genotoxic, they may also have been clastogenic to soil organisms especially benz(b)quinoline. These differences might be useful in soil contamination / risk assessment procedures and also provides basis for regulatory action.

MO 273

Effects of the use of onshore drilling cuttings as soil amendment on reproduction of Eisenia andrei
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The onshore procedures of an oil drill well generate a great amount of drilling cuttings that are composed of rocks, sand, oil residues and drilling fluid. The cuttings volume varies between 0.1 to 0.5 barrels of oil per ton of drilled rock, and depends on the geologic character of the cuttings. The drilling cuttings are treated to recover the fluid, using different equipment, as an alternative cleaner and energy source. A common drilling fluid in Brazil contains barium sulfate (Ba SO4), what requires an appropriate disposal or safe utilization. One alternative use is as soil conditioner through the amendment of acid soils. However, the cuttings have other potential contaminants as arsenic, lead, molybdenum, zinc and sodium that can act in a synergistic harmful way to soil organisms. The objective of this study is to test mixes of drilling cuttings and soil, to determine the potential ecotoxic effects of this residue to soil non-target organisms. Reproduction assays with Eisenia andrei were performed using two types of cuttings: from the drier and from centrifuge, with artificial OCDE soil (10% organic matter content). The barium doses tested were: 300, 900, 1500, 2100, 2700 e 3000 mg Ba.soil kg-1, which correspond to critical values determined by Brazilian law. Results demonstrated that the waste from the cuttings drier (EC 50 = 114 mg Ba.soil kg-1) had a more deleterious effect to Eisenia andrei reproduction than the waste originated in centrifuge (EC 50 = 797 mg Ba.soil kg-1). A more detailed ecotoxicological study has to be carried out to ensure the environmental safety of the cuttings as soil conditioner in agriculture.

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MO 274
Methodological improvements in the study of pollutant-induced toxicity on the earthworm Eisenia andrei: the effects of chromium(VI) on coeloocytes physiology
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Over the last decades, the widespread use of chromium by industries has led to numerous cases of environmental contamination by this element. In mammal cells, Cr(VI) is reduced to Cr(III) during which various reactive oxygen species (ROS) are generated. Cr(VI) is widely known to cause toxic and genotoxic effects in vertebrates. Relatively less investigated are the effects of Cr(VI) on invertebrates, particularly on the ecdysropic organisms. In vitro and in vivo studies were performed on Eisenia andrei to investigate the biological effects of environmentally relevant concentrations of Cr(VI). In this study, we show the results obtained on coelomocytes of earthworm exposed for 1 and 3 days to different sublethal concentrations of Cr(VI) using the filter paper test. Coelomocytes are the cells circulating in the coelomic fluid and playing an important physiological role in the immune defence of earthworms. Cr(VI)-induced oxidative stress was investigated by evaluating the radical production using fluorescent probes, the lysosomal accumulation of lipofuscin and the oxidative damage to DNA. Moreover, to highlight changes in cellular physiology, mitochondrial membrane potential, lysosomal membrane permeability, pathological reactions involving the lysosomal system are often linked to augmented autophagic sequestration of cellular components. In situ FITC diaactate labeling of intracellular proteins was performed to assess the transport into autophagocytic degradative compartments of these proteins. In addition, the leakage of lactate dehydrogenase in the coelomic fluid was determined to reveal cell membrane damage. Finally, the immune response of coelomocytes consisted in the phagocytic uptake and in the significant changes in the different parameters evaluated on coelomocytes of treated earthworms. Cr(VI) provoked, just 1 day after the exposure to lower doses, an increase in ROS levels, generating in the cells oxidative stress conditions leading to lipid peroxidation with lysosomal lipofuscin accumulation and oxidative damage to DNA. Cr(VI) also caused alterations of intracellular compartments until to reduce, at the highest dose, at the longer exposure time, the immune response of the cells. Overall, the data demonstrated that oxidative stress is an important factor involved in Cr(VI)-induced toxicity and genotoxicity in E. andrei coelomocytes.

MO 275
An exposure regime that brings more realism in the testing of plant protection products in soil
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A modified exposure regime for Plant Protection Products (PPP) in soil eco-toxicological test systems (e.g. earthworm, collembo, or predatory mite reproduction test) is proposed in order to achieve more realism in the risk assessment. Beside active substances, the toxicity of PPP is often influenced by highly volatile solvents in formulations (i.e. in emulsion concentrates), which degrade fast and occur only in the top soil layer. In the risk assessment the Predicted environmental concentrations in soil (PECsoil) are compared to the endpoint in the laboratory test and a safe use is indicated if an assessment factor of 5 is surpassed. For persistent active substances (a.s.) the PECsoil consists of the plateau concentration (only the a.s.) and the initial concentration (the a.s. within the product). However the potential effects of the formulation inerts within the end use product can be limited in space and time. Thus, the risk of PPP to soil receptors (i.e. the NOEC) is related to a PECsoil calculated for 0–1 cm soil depth and b) if the end use product is evenly distributed in the soil layer to establish the test concentration in the laboratory test. In this study we examine the effects of a PPP (fungicide; EC formulation with 2 persistent a.s.) on the reproduction of Eisenia fetida (OECD, 222) will be determined according to the current test design and compared to a more realistic exposure regime considering a plateau concentration regime consisting of the plateau concentration (only the a.s.) and the initial concentration (the a.s. within the product) at 0–5 cm soil depth. The test organisms are inoculated and the end use product is applied by overspray application. Finally, the total concentrations of the a.s. in the treatment groups can be calculated as the sum of two components: a) the plateau concentration and b) the calculated concentration from the overspray application, considering a depth of bulk soil of 0–1 cm and a soil density of 1.3 g/cm³ for 1 and 3 days exposure time. This modified test design considers two aspects improving the realism of the exposure of PPP in soil eco-toxicological test systems: 1) a more realistic distribution of soil organisms to formulation inerts and 2) a more realistic distribution of the PPP in the uppermost top soil layer, considering the specific sorption properties of the a.s. This modification would improve the realism without losing the conservativeness necessary in a tier I test system in the registration process for PPP.

MO 276
Introducing the ‘ResOrgRisk’ Project: environmental risk assessment of the use of organic residues as soil amendments
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The amount of organic residues (ORs) generated worldwide is increasing and environmentally sound strategies for their disposal and recycling must be developed and optimized. Council Directive 1999/31/EC has set down strict mandatory targets for reduction in the amount of ORs reaching landfill; by keeping organic matter away from landfills, their nutrients can be recycled, less methane is emitted at the landfill, and leachates produced are less polluted. Recycling ORs (e.g. sewage sludge, compost, agro-food residues) in agriculture is considered a way of maintaining or restoring the quality of soils, enlarging the slow cycling soil organic carbon pool. However a wide variety of undesired traits, such as heavy metals and organic contaminants, can have adverse effects on the environment. In fact, the Proposal for a Soil Framework Directive recognized that “soil degradation or soil improvements may have a major impact on other areas, […] including surface waters and groundwater, human health, climate change, protection of nature and biodiversity, and food security”. Taking that into account, the aim of the Project “ResOrgRisk” is to assess the environmental risk of the use of ORs as soil amendments, evidencing their benefits and constraints, and emphasizing which uses are more trustworthy to assess their quality and the environmental risk of their application.

The study will rely on integrated results obtained by a multidisciplinary team based on:
1. Chemical, biochemical, microbial and ecotoxicological characterization of the ORs, of soils amended with ORs, and of their leachates/extracts;
2. Ecotoxicological and ecological evaluation of effects and fate of pollutants derived from the application of different ORs to agricultural soil using TME’s (Terrestrial Model Ecosystems) experiments;
3. Determination of chemical, biochemical, microbial, ecotoxicological and ecological effects of the application of ORs to soils in field experiments.

Classical and instrumental chemical analysis will be used to quantify physico-chemical characteristics of the materials. Ecotoxicity tests will be used to provide an integrated measurement of both environmental and toxicological bioavailability of the contaminants, and their detrimental effects in the ecosystem. The effects in soil microbial communities will be assessed evaluating microbial indicators, and using molecular methodologies.

MO 277
Validation of the Czech directive 257/2009 Coll. determining the limits for the defended limits for applied on agricultural soils. There are also 4 ecotoxicological bioassays suggested for the directive. In our study, we verified the validity and reliability of the selected test batteries using thirty-six sediment samples and wide battery of ecotoxicity bioassays, both for solid and eluate samples. The aim was to compare directre bioassays with other soil bioassays (for example, faster and more practical ones) and to compare bioassays of eluates with the soil bioassays.

MO 278
Selection of relevant ecotoxicity tests to assess the effects of biosolids used in agriculture
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In the last decade, and in accordance with environmental sustainability, biosolids are applied on fields. These materials are used as fertilizers, to maintain a high production rate of plants. If they are not a source of nutrients, they can be potentially a source of pollutants. As a consequence they can affect plants, soil fauna and finally human health. In this study, environmental effects of different biosolids (sewage sludge, manure, composted materials [3DOTS]) were studied using ecotoxicity tests on soil and water. The aim of this work was to establish a battery to assess the effects of biosolids, by comparing their respective sensitivities towards these materials. Organisms composing the biotest battery and their respective acute and/or chronic endpoints are the following:
- algal growth inhibition of Pseudokirchneriella subcapitata (NF EN ISO 8692)
- inhibition of the immobilization of the rotifer Brachionus calyciflorus (NF ISO 6341)
- inhibition of light emission of the bacteria Vibrio fischeri (NF ISO 20666)
- inhibition of root growth, emergence and growth of higher plants [ISO 11269-1 and ISO 11269-2]
- avoidance and reproduction inhibition of the earthworm Eisenia fetida (ISO 17512 and ISO 11126-8)

All of these bioassays were conducted using their respective standardized protocol, except for C. elegans soil tests which were carried out using the method detailed in another work (Hugosier et al., 2013). The cell viability and reproduction test to assess the effect of solid wastes; same conference topic). Concerning samples preparation, standard soils (ISO and/or LIFA) were mixed with biosolids at defined doses (application rate, and e.g. 2 times, 5 times, 10 times, 20 times the...
This study was funded by Spanish projects CTM2010-19779-C02-01/02 and RA2010-00004.

MO 280

Analytical and ecotoxicological characterization of biosolids collected from Spanish wastewater and municipal solid waste treatment plants

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Biosolids are end products of wastewater and municipal solid waste (MSW) treatments processes and are, therefore, rich in nutrients and organic matter. Regulations on the presence of pathogens, nutrients and metals in biosolids for use as agricultural soil amendments are clear; however, the contents of organic compounds, such as pharmaceutical, personal care products (PCPs), endocrine disrupting compounds (EDCs) or persistent organic pollutants (POPs), are not yet regulated. Indeed, Spain and other EU Member States have considering the two European Directives on applications of composted MSW (Directive 86/277/EEC) and sludge WWTPs (2nd Draft of Biological Treatment of Biodegradable Waste, 2001) in soil for agricultural purposes to establish new limits for the pollutants first considered and to increase the number of regulated substances as regards new emerging organic compounds. As a consequence, the presence of potential environmental risk of biosolids is necessary to know their chemical composition and their effects. This study aims to obtain information about the analytical and ecotoxicological assessment of 16 biosolids collected from different Spanish wastewater and MSW treatment plants using previous step tests such as in-vitro microcosms and field studies. The results of the characterized biosolids show relevant differences among the samples. The results obtained so far do not indicate genotoxic effects with the Comet assay and in addition, toxicity of sediment samples was experimentally assessed.

MO 281

State of the art soil ecotoxicity standards in Brazil: challenges and perspectives

L.S. Nascimento

Soil ecotoxicology in Brazil had a conceptual mark in 1988 when it was published by the extinsted Special Office of Environment (SEMA) the Handbook of Tests for the Ecosafety Evaluation of Chemicals. In that document it was described a single method for soil organisms toxicity evaluation: a method (Norm X31-250, 1984) using earthworms (Eisenia fetida) based on AFNOR (ASSOCIATION FRANCAISE DE NORMALIZATION). This method was applied during several years for pesticides registry and the study of pesticides ecotoxicity was also the major interest in soil ecotoxicology of the Brazilian researches during the last years. In 2002 it was created the Special Committee of Ecotoxicological Analysis of ABNT (Brazilian Association of Technical Standards), an organization member of ISO - International Organization for Standardization - responsible for the development of Brazilian Standards in many areas. The Committee of Ecotoxicological Analysis was created based on the increasing demand related to quality and new tests, for example, using soil organisms. In 2004 an increasing in soil ecotoxicological studies was noted in Brazil mainly because of the raising of academic and research interest and partnership with Germany and Portugal. So, in 2006 ABNT wrote the first Brazilian Standard related to terrestrial ecotoxicology and soil quality (ABNT NBR 15537:2007, acute toxicity test with earthworm), based on the translation of ISO 11268-1:2003. Nowadays there are six Brazilian Standards published (tests with earthworms, collembola, and soil flora; guidance for ecotoxicological characterization of soil and soil materials) based on ISO Standards (ISO 11268-1:2003, ISO 17112-1:2008, ISO 11267:1999, ISO 11259-2:2005, ISO 17616:2008, ISO 15799:2003). The work will continue and the group intends to translate ISO Standards that could be applied on contaminated sites, an increasing problem in Brazil. These standards will be the basis for the development of new researches and tropical findings that would be incorporated in the next revisions. The members of ABNT Committee of Ecotoxicological Analysis believe that this is just the first step because a lot of work need to be done but the availability of standards written in Portuguese and acquired with local money could be an important way for the development of soil ecotoxicology studies in Brazil.
The impact of red mud on reproduction of the collembolan *Folsomia candida*  
M.O. Bianchi, M.E.F. Correia, A.S. Resende, E.F.C. Campello, J.P. Sousa  
INEM-CMA, University of Coimbra, Coimbra, Portugal  
Red mud is a residue generated from the refining of bauxite for alumina production. The concentrations tested were: 0, 2.5, 5, 10, 20, 30 and 40% of residue (w:w basis). Results showed that the total metal and arsenic concentrations showed high heterogeneity among samples with most points along the CA exceeding the benchmark values. Results showed that the high sodium content (Na\(^+\) = 49.19 cmol, dm\(^{-3}\)) is probably the most deleterious factor to *Collembola* reproduction. The EC50 was 9.36% for OCDE and only 1.36% for natural soil. This difference indicates that the presence of the natural soil increase the toxicity of the red mud and that strong protective measures should be taken when encapsulating this residue.

MO 286  
Assessing biological activity of soil organisms in no-till soils under different agricultural practices using the bait lamina method  
S. Campiche, C. Maurer-Troxler, I. Werner  
Ecotoxic Centre Eawag/EPFL, Lausanne, Switzerland  
Müllermuseum und Naturkunde Museum Bern, Bern, Switzerland  
Schweizerisches Zentrum für angewandte Ökotoxikologie Eawag/EPFL, Dübendorf, Switzerland  
In agriculture, application of fertilizers and phytosanitary products varies depending on the cultivation methods used. Sustainable farming practices such as zero tillage increase the use of organic matter in the soil. This work aims to study the impact of different fertilizer and herbicide treatments on soil organisms. The objective of this study is to test mixtures of red mud and soil to provide information about the potential toxic effects of this residue to soil non-target organisms. Reproduction assays with *Folsomia candida* were performed using two types of mixtures: with artificial OCDE soil (5% organic matter content) and with a natural soil from Fazos de Macopin municipalities, Minas Gerais state, Brazil. The concentrations tested were: 0, 2.5, 5, 10, 20, 30 and 40% of residue (w:w basis). Results demonstrated that the red mud and its mixtures with artificial OCDE soil and natural soil are moderately toxic for *Folsomia candida*. No juveniles were observed at concentrations of 30% of residue for red mud and 10% for mixtures, while no or very few juveniles were found at the remaining concentrations. The number of juveniles of *F. candida* was lower than the control at all plots but almost zero at 11 out of 15 plots. These results are probably caused by a complex interaction between soil parameters, contaminants, and the salt content.

MO 287  
Effects of contaminated soils from North Africa on enthyraeids and predatory mites in standard laboratory tests. II. The case of a Phospopygum plant near Sfax (Tunisia)  
J. Rodriguez-Villalon, M. Märker, A. Haddioui, M. Kolti, R. Pereira, J.P. Sousa  
Faculty of Agriculture, University of Porto & University of Aveiro, Portugal  
University of Coimbra, Coimbra, Portugal  
University of Sultan Moulay Slimane, Beni Mellal, Morocco  
The results of the reproduction assays with *Folsomia candida* are discussed in the previous paper (MO 286). The present work, the impact of different fertilizer and herbicide treatments on soil organisms, of a no-till soil was compared. Using the bait lamina test, the changes in overall feeding activity of the soil biocenosis were measured in agricultural brown soil (5% clay, 3% humus) with and without application of glyphosate for two types of fertilizers. The experiment was conducted at different exposure times (2h, 8h, 24h). The results show that the number of juveniles and the feeding activity of the herbicide application, at different application rates and with different crops (cotton, winter barley). The results of the bait lamina test show that according to the exposure parameters and application rate of the herbicide, the biological activity of the soil organisms tends to be higher in soil where glyphosate was applied. The bait lamina results were completed by physico-chemical analyses and by reproduction tests with Collembols on the collected field samples.

MO 288  
Assessing pesticide impacts on soil compartment at a Brazilian potato and strawberry producer region  
Escola de Engenharia de São Carlos/Universidade de São Paulo, São Paulo, Brazil  
University of São Paulo, São Carlos, Brazil  
University Federal de São Carlos, São Carlos, Brazil  
*Petromar, São Carlos, Brazil*  
Interviews with potato and strawberry producers from Bom Repouso, MG, Brazil, showed intense and inappropriate utilization of pesticides. Local soil samples presented residue of aldrin, heptachlor, heptachlor epoxide, chlorpyriphos and carbofuran (potato soils); aldrin, heptachlor epoxide, endosulfan-I, carbofuran and abamectin (strawberry soils). Using the bait lamina test, the changes in feeding activity and reproduction of the soil biocenosis were measured in agricultural brown soil (15% clay, 3% humus) with and without application of abamectin. The experiment was conducted at different exposure times (2h, 8h, 24h). The results show that the number of juveniles and the feeding activity of the herbicide application, at different application rates and with different crops (cotton, winter barley). The results of the bait lamina test show that according to the exposure parameters and application rate of the herbicide, the biological activity of the soil organisms tends to be higher in soil where glyphosate was applied. The bait lamina results were completed by physico-chemical analyses and by reproduction tests with Collembols on the collected field samples.
The aim of this publication is to compare different sampling schemes and their potential for the evaluation of micro-arthropod communities in field trials. Sampling resilience (last pesticide applications occurred in August) was estimated. Arthropods were sampled during fall, spring and summer in 20 apple orchards with different management strategies using six pitfalls in each orchard. We concluded that not all arthropod families have the same sensitivity to pesticide management. The next step is to determine if difference in sensitivity occurred as well for other soil invertebrates. Arthropods are a primary endpoint in field studies and it is important to have an idea about seasonal population changes over the period of a year. In this publication the seasonal changes and abundance of micro-arthropod communities in two fields are presented over one year. Results are discussed in terms of test design and sampling scheme.

Effects of different land uses on soil microarthropod biodiversity in a Mediterranean urban context

The majority of human activities frequently lead to the conversion of natural habitats to anthropogenic land uses. Land-use change is an important form of global pressure affecting biodiversity. Unfortunately, little is known about causes and consequences of soil biodiversity loss, although soil biodiversity affects the main regulatory services of the ecosystem. The present study aimed to analyze biodiversity of soil microarthropods in soils with different human impact, in order to highlight how different land-use affects soil arthropod communities. To evaluate if different land uses cause differences in kind and degree of pollution, the content of metals and PAHs were related to different taxa of soil arthropods to individuate the taxa more sensitive/tolerant to different pollutants. To this purpose, 18 soil samples, grouped in 4 different categories (natural, residential, industrial and agricultural), were collected in Naples and surroundings (Southern Italy). The main soil chemical characteristics: water holding capacity, bulk density, organic matter content, content of nutrients, metals, PAHs, were analyzed. To evaluate the arthropod biodiversity, the organisms were extracted using Macfadyen method. Abundance of Mites (identified at the sub-order level) and abundance and diversity of Collembola (identified at the species level) were assessed. Preliminary results highlighted that two taxa (Collembola and Acarina) were extremely widespread and abundant in all the investigated soils. Arthropod communities largely differed among the soil typologies.

Year-round responses of soil invertebrate communities to pesticide applications in Mediterranean apple orchards

Since the abundance of micro-arthropods is a primary endpoint in field studies it is important to have an idea about seasonal population changes over the period of a year. In this publication the seasonal changes and abundance of micro-arthropod communities in two fields are presented over one year. Results are discussed in terms of test design and sampling scheme.

Effects of the nitramine explosive CL-20 on soil invertebrate communities in a sandy loam soil

The soils were analysed for physical-chemical properties (pH, water holding capacity, bulk density, porosity, organic matter content, content of nutrients, metals, PAHs). To evaluate the structural and functional protection goals for soil given in the EU directive for the registration of plant protection products. In the past the impact of a plant protection product on the soil's ability to turn over organic matter was tested with litter bag studies. Recently the structure of the micro-arthropod community has been the focus for a future directive.

The main challenge for field tests with micro-arthropods is the agroecosystem. We test the protection goals for soil given in the EU directive for the registration of plant protection products. In the past the impact of a plant protection product on the soil's ability to turn over organic matter was tested with litter bag studies. Recently the structure of the micro-arthropod community has been the focus for a future directive.

What to do when there are too many specimens: two strategies for sub-sampling in Off-crop studies for non-target arthropods

Microarthropod communities in lead contaminated forest soils at active and abandoned shooting ranges

As a part of a larger ecosystem-scale investigation, microarthropod communities at two shooting range sites with different contamination history and at one uncontaminated site were studied. The three study sites locate in a same pine forest ecosystem; one site situates right behind an active shotgun shooting range and another site locates in a forest. As a part of a larger ecosystem-scale investigation, microarthropod communities at two shooting range sites with different contamination history and at one uncontaminated site were studied. The three study sites locate in a same pine forest ecosystem; one site situates right behind an active shotgun shooting range and another site locates in a forest.
Over the last four years protocols have been developed to test the influence of plant protection products on arthropods in off-crop areas. However, for some sampling methods, e.g. suction sampling, in off-crop areas high numbers of arthropods together with high amounts of undesired material (e.g. fine plant material, seeds, litter and soil) are sampled at the same time. This leads in turn to a tremendous work load for sorting and taxonomical determination of the specimens, which rapidly exceeds an acceptable time period for practical and economical purposes. Therefore, procedures need to be developed to efficiently and effectively use the samples with high specimen numbers. Here, two different sub-sampling methods were used for Vortic serotonin samples from an off-crop field study with high individual numbers (e.g. > 15 000 individuals per sample). The first method uses a sample divider in four steps. The divider is originally constructed for creating sub-samples of plankton samples. The second method was additionally used to set a time limit for sorting the arthropods that are not micro arthropods out of the sample. The aim was to take at least 80% of the arthropods out of the sample before the sub-division. As a following step the sub-divided samples were divided by the sample divider before counting the numerous collembolans and mites. To assess the performance of both methods the coefficient of variance for different taxa identified was calculated and compared. There was also an assessment of the sample time for the two methods.

**MO 297**

Computer controlled high gradient extractor for soil organisms

G. Henkes
RIFCON GmbH, Hirschberg, Germany

Symbiosis is poorly understood. This is especially true for new and modern active compounds. First results of laboratory experiments presented on the poster were performed to choose fungicides to be used in field experiments.

Studies have shown that fungicides on the one hand stimulate and on the other hand inhibit mycorrhization [2,3] . However, the impact of fungicides on the mycorrhizal symbiosis is poorly understood. This is especially true for new and modern active compounds. First results of laboratory experiments presented on the poster were performed to choose fungicides to be used in field experiments.

**MO 298**

Use of anaerobic digested-based composts as soil organic amendments: effect on the soil biological status

EAS EcoChem GmbH, Nienfenn-oeschelbronn, Germany

Over the last four years protocols have been developed to test the influence of plant protection products on arthropods in off-crop areas. However, for some sampling methods, e.g. suction sampling, in off-crop areas high numbers of arthropods together with high amounts of undesired material (e.g. fine plant material, seeds, litter and soil) are sampled at the same time. This leads in turn to a tremendous work load for sorting and taxonomical determination of the specimens, which rapidly exceeds an acceptable time period for practical and economical purposes. Therefore, procedures need to be developed to efficiently and effectively use the samples with high specimen numbers. Here, two different sub-sampling methods were used for Vortic serotonin samples from an off-crop field study with high individual numbers (e.g. > 15 000 individuals per sample). The first method uses a sample divider in four steps. The divider is originally constructed for creating sub-samples of plankton samples. The second method was additionally used to set a time limit for sorting the arthropods that are not micro arthropods out of the sample. The aim was to take at least 80% of the arthropods out of the sample before the sub-division. As a following step the sub-divided samples were divided by the sample divider before counting the numerous collembolans and mites. To assess the performance of both methods the coefficient of variance for different taxa identified was calculated and compared. There was also an assessment of the sample time for the two methods.

**MO 299**

Evaluation of interactive toxic effects of binary mixtures to the bioluminescent fungus Gerronema viridilucens

F.M. Ventura, L.F. Mendes, E.L. Bastos, C.V. Stevanin
Universidade de São Paulo, São Paulo, Brazil

Fungal-based assays can be used to assess the toxicity of environmental samples of polluted areas. Toxicity studies with binary mixtures of metals are more realistic than those with single metals as interactions between the species can interfere with the toxicities. Currently, there is no work on the combined toxic effect of metal binary mixtures to fungi, despite the importance of these organisms in soil nutrient cycling. This work reports the use of a fungal-based luminescent assay to test the toxicity of binary mixtures of metal cations (Ca
+2
, Cd
+2
, Cu
+2
, Zn
+2
). The toxic unit concept was used to calculate the EC50 values of binary mixtures (EC50
mixC
). Experimental EC50 values (EC50
mixA
) were obtained from bioluminescence inhibition vs. log(cation concentration) curves and were compared with EC50
mixA
 to determine the nature of interactions (antagonistic, additive, greater than additive, or synergistic). The single metal cation toxicity was found to be Ca
+2
>Cu
+2
>Zn
+2
> Cd
+2
. The interactive effects were as following: synergistic for Cu
+2
+Zn
+2
, betweel, Monasterito, and greater than additive for Ca
+2
+Zn
+2
, between additive and greater than antagonistic for Cu
+2
+Zn
+2
+ Cd
+2
, and antagonistic for Ca
+2
+Cu
+2
.

**MO 300**

Crude oil pollution on land can cause serious damage to soil by affecting both its biophysical and biochemical properties. Microbial populations (and microbial diversity) in soil provide adequate nutrients and stored energy (ATP) for primary productivities. Hence, in this study, the effect of terrestrial oil pollution on the ATP content of the soil and the soil micro-arthropods in our territory were determined. Soil samples were obtained from a large area near the city of Rome (Lazio, Italy). To assess the performance of both methods the coefficient of variance for different taxa identified was calculated and compared. There was also an assessment of the sample time for the two methods.

**MO 301**

Advantage of fungicides on ectomycorrhizal mycelium growth - impact of quinoxone, boscalid, tebuconazole and oxazosobrin

K. Zunker, W.U. Palm, W.K.L. Ruck
Leuphana University Lüneburg, Lüneburg, Germany

Ectomycorrhizal fungi (EM) are symbiotic mutualistic tree species such as European beech (fagus sylvatica L.). In Germany about one third of the landscape is forested, the European beech dominating large areas. Mycorrhized plants have an increased and thereby more efficient water and nutrient uptake and are better adapted to the specific content of the amended soil. Hence soil ATP can be used as a reliable marker to assess the impact of crude oil pollution on soil ecosystem productivity and microbial diversity.

Use of anaerobic digested-based composts as soil organic amendments: effect on the soil biological status

F. Ventura, L.F. Mendes, E.L. Bastos, C.V. Stevanin
Universidade de São Paulo, São Paulo, Brazil

Crude oil pollution on land can cause serious damage to soil by affecting both its biophysical and biochemical properties. Microbial populations (and microbial diversity) in soil provide adequate nutrients and stored energy (ATP) for primary productivities. Hence, in this study, the effect of terrestrial oil pollution on the ATP content of the soil and the soil micro-arthropods in our territory were determined. Soil samples were obtained from a large area near the city of Rome (Lazio, Italy). To assess the performance of both methods the coefficient of variance for different taxa identified was calculated and compared. There was also an assessment of the sample time for the two methods.

**MO 302**

Advantage of fungicides on ectomycorrhizal mycelium growth - impact of quinoxone, boscalid, tebuconazole and oxazosobrin

I. Zunker, W.U. Palm, W.K.L. Ruck
Leuphana University Lüneburg, Lüneburg, Germany

Ectomycorrhizal fungi (EM) are symbiotic mutualistic tree species such as European beech (fagus sylvatica L.). In Germany about one third of the landscape is forested, the European beech dominating large areas. Mycorrhized plants have an increased and thereby more efficient water and nutrient uptake and are better adapted to the specific challenges of climate change, such as drought stress [1]. Studies have been performed on the further development and on the other hand inhibit mycorrhization [2,3]. However, the impact of fungicides on the mycorrhizal symbiosis is poorly understood. This is especially true for new and modern active compounds. First results of laboratory experiments presented on the poster were performed to choose fungicides to be used in field experiments. The impact of authorized fungicides used in agriculture and forestry on the ECM fungi psilolithus arthusii, hebeloma crustuliniforme, lyophyllum and cenecocum geophilum was investigated. In a first step active ingredients in quinoline, boscalid, tebuconazole and oxazosobrin were examined in in-vitro experiments under laboratory conditions. In a consecutive step corresponding commercial fungicide formulations were used. For each formulation one dilution was prepared from which 150 ml were used for each dilution. The dilutions were applied to agar plates and the agar contents were determined at the different dilutions. The experiments were conducted in Petri dishes as well as with sterilized soils to approximate natural conditions. For the plate tests mycelium was taken and placed on the treated medium. The test tubes were incubated for 29 days at 25°C in the dark. The evaluation and quantification was performed by determination of the radial growth of mycelium corrected by results from the control group. In 29 days the mycelium caused the strongest inhibition whereas the lowest inhibition of mycelial growth was found for quinoxine. For Psilolithus arthusii an inhibition was found even at 0.1 % application rate for three fungicides. For Cenecocum geophilum an inhibition was found at 10% application rate using tebuconazole. Interestingly an irreversible damage of ECM was found. Hence, ECM with inhibited growth after 29 days shows adverse effects on mycelial growth even after re-inoculation on untreated control medium.

MO 302
Selective removal of high molecular weight PAHs in crude oil and tar contaminated soils using biodiesel
T.O. Oritake, D.M. Jones
Newcastle University, Newcastle upon tyne, United Kingdom

An observation that biodiesel amendment can enhance the selective removal of benzo(a)pyrene has been made in soils spiked with crude oil and coal tar. Results from laboratory microcosm experiments of tar spiked soils showed that after 60 days, this pollutant was reduced by 91% in biodiesel amended samples as compared to 17% and 29% reductions in the control and nutrient amended microcosms, respectively. Perylene was reduced by 70% and anthracene was almost completely mineralised in the biodiesel amended experiments. However, there was no detected depletion in phenanthrene in the biodiesel amended soils as opposed to that in the control (91%), and nutrient amended (85%) experiments, even up to a 180-day degradation period. Fluoranthene and pyrene were also not depleted in the biodiesel amended experiments. A similar removal trend was observed for the crude oil spiked experiments which showed 52% removal of benzo(a)pyrene which was present in the biodiesel amended samples after 60 days. However, the degradation of fluoranthene, pyrene, and phenanthrene was substantially inhibited in these samples as compared to the other treatments. The six ring compounds indeno[1,2,3-cd]pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene were observed to be more degraded in the treatments with biodiesel for both crude oil and tar contaminated soils.

Further work to investigate this process and also soil toxicology changes with treatments are being conducted.

MO 303
The contribution of bacterial cell wall fragments to the formation of soil organic matter - a case study from a glacier forefield
C. Schürg1, R. Smittenberg1, J. Berger2, F. Kral1, S.K. Woche2, M.O. Gobel1, H.J. Heipieper1, A. Mittler1, M. Kästner1
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Max Planck Institute for Developmental Biology, Tübingen, Germany

Overall 51 soil samples were collected of the Mashavera valley in March 2010. Habitat quality was evaluated by determination of pH-value, organic matter, soil texture and proximities to the factory. In highly polluted smelter locations the decomposition of litter and thus nutrient cycling is impaired due to the almost complete absence of litter decomposers in the soil and litter layer. Endemic soil microbial communities vary from site to site; therefore a set of tests with different assessment endpoints is needed. The aim of the study was to test the use of soil microbial parameters for the evaluation of the habitat function of soils contaminated with trace metals.

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Pre-investigations based on Koh Log bioassays have been attempted, but these are not very reliable, especially for ionic compounds, such as many APIs of interest. Therefore, better predictive models are urgently needed that include binding mechanisms in addition to the hydrophobic interaction. As activated sewage sludge is a complex matrix and hard to fully characterize we used ten commercially available solid phase extraction (SPE) cartridges with different chemistries to try to reproduce these binding processes. Various eluents with different organic modifiers and different buffers at a range of pH were tested. The results of these experiments, where the sorption mechanism was known, were compared to results obtained using ‘in house’ prepared cartridges of sludge at different sludge/silica ratios.

MO 308

Risk assessment of pesticides to soil organism: exposure analyses of two insecticides in soil layers during a sampling period of one year

C. Fossberg, B. Schmidt, A. Schaefter

RWTH Aachen University, Aachen, Germany

The spatial and temporal behavior of pesticides in the soil environment depend on properties of the substance (e.g. degradation, leaching, sorption), the soil and the climate.

To study the effects of the chemicals on the soil fauna, the spatial and temporal distribution of soil organisms is also important. Within the project “Risk assessment of pestiticide effects of rape under real conditions” we investigate the biodiversity of soil organisms. Thus far, invertebrates, such as earthworms, nematodes, and mites, have been studied. We are using earthworms (Eisenia fetida) as indicators due to their high level of sensitivity to contaminants and the fact that they can easily be reared in the laboratory.

Bioremediation, namely the nature of soil contamination (spiked-phenanthrene and pyrene each at 100 mg/kg dry soil), bioaugmentation (with a PAH-degrading microflora) and planting (with Miscanthus x giganteus), were arranged according to a full factorial design in a pot experiment. Their effects (alone or in combination) were assessed on soil organisms. Two insecticides, imidacloprid and lindane, both persistent in soil, but differing in the leaching behavior were separately applied to Terrestrial Model Ecosystems (TMEs) in two concentration each: 7,5 kg/ha and 20 kg/ha (lindane) and 0,75 kg/ha and 2 kg/ha (imidacloprid). The distribution of the pesticides in the soil profile (0,25 cm, 2,5 cm, 5 cm, 10 cm and 10-20 cm) was determined using GC/(MSMS).

We here present the analytical work. Lindane was extracted with ethyl acetate and the residues were determined by gas chromatography with electron-capture detection. Imidacloprid, a neonicotinoid, was extracted by microwave digestion in acetonitrile/water; after centrifugation the extract was analyzed with LC/MS/MS. Results of the currently performed chemical analyses will be presented. In another part of the project the effect of the insecticides to soil organisms on the population level are investigated and shown in a platform presentation.

MO 309

Understanding the relationship between extraction technique and bioavailability

C.V. Eadsforth1, M. Galay Burgess2, M.S. Holf2, D.J. Letskin1, H. Noble1, R. Oliver2, M. Telscher3

Shell International Ltd., Shell, United Kingdom

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AstraZeneca, Brentwood, United Kingdom

Syngenta, Guildford, United Kingdom

Bayer CropScience, Monheim, Germany

In addressing the significance of bound residues in environmental risk assessment, ECETOC concluded previously that non-extractable residues were characterized by a pragmatic approach for determining whether they are extractable or not. There is now a need to develop a standard framework for soil and sediment extraction methods and to associate extractable fractions (such as freely dissolved, readily desorbed or reversibly desorbed) with a level of bioavailability and with appropriate test organisms for these compartments. A Task Force has reviewed extraction methodologies and how such methods correlate with bioavailability, identified those ‘destructive’ extracts that are currently used and recommended a role for these results in loss of sample matrix integrity and also considered the state of knowledge regarding mechanisms of binding and their forces which would result in either extractable or non-extractable residues. The aim was to develop new approaches to screen the bioavailable fractions and validate such an approach. This supports a consistent interpretation of data and provides a basis for assessing the potential risk of non-extractable residues. The Task Force will evaluate a framework for an intelligent extraction strategy and make recommendations for future research topics.

MO 310

Plant uptake of some commonly used pharmaceuticals

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Presence of different pharmaceuticals in sewage sludge is apparent, but there is still a lack of knowledge about the fate of pharmaceutical residues in the environment. Still, remarkable amounts of pharmaceuticals enter the soil via fertilization with sewage sludge. As long as such a disposal practice is widely applied, a better knowledge on the fate of pharmaceuticals in sludge treatment processes is necessary. Only a relatively small number of investigations has been published on the mobility and bioavailability of pharmaceuticals in the environment. Uptake of antimicrobials into vegetation is a major route for these substances into the food chain. In the current study uptake of ciprofloxacin, norfloxacin, ofloxacin, sulfamethoxazole and sulfamethoxazole was demonstrated in the case of lettuce, potato, carrot, and wheat. Measurable residues of the antimicrobials in the food plants were found. The highest concentrations of these pharmaceuticals in fruits and plants appears to be caused by the plants' high health risk, as the detected levels of the studied pharmaceuticals were of considerable magnitude, if compared to their soil concentrations. As a rule, the uptake of antimicrobials was more efficient from sandy soil into underground parts on plants - roots and tubers. The concentrations of antimicrobials in plants exceeded the MRL values for meat and milk were not exceeded at antimicrobial soil concentrations equal to 1 mg/kg and 10 mg/kg. Such concentrations may be present in soil if sewage sludge treated by anaerobic digestion is used as a fertilizer. In Estonia the soil concentrations of antimicrobials are presumably lower, as only sewage sludge compost may be used for fertilization.

MO 311

Degradation of pharmaceuticals in sewage sludge compost

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Sewage treatment facilities do not remove pharmaceutical residues completely. Several antimicrobials do not decompose during aerobic activated sludge process or anaerobic digestion, and release in sewage effluents or adsorb to sewage sludge. The sewage sludge containing pharmaceutical residues is used as a fertilizer. Pharmaceuticals reach the soil where they can affect microorganisms and accumulate in plants. Our recent study showed that some fluoroquinolones and sulfonamides were frequently present in Estonian sewage sludge and the composting technology played the major role of their degradation. In Tallinn, where composting with peat was used and mixing of the compost stacks took place during 8 months, the highest contents of ciprofloxacin, norfloxacin, ofloxacin after a year storage were 70, 64 and 8 µg/kg (dm) respectively. Fluoroquinolones have been described as photodegradable, therefore we suppose that the light due to frequent and long lasting mixing could be one of the reasons of their faster decomposition in Tallinn. Plant experiments in greenhouse have shown that potato, carrot and lettuce can uptake fluoroquinolones from soil with antimicrobial concentration 10 µg/kg (dm) (the trigger value for antimicrobial content in soil recommended by EMA/CVMP). The mixture of sulfonamides and fluoroquinolones in soil can be toxic for wheat and lettuce plants, if antimicrobial concentration in soil reaches 10 mg/kg (dm). Antimicrobial content in plants grown at soil antimicrobial concentration 10 mg/kg exceeded the MRL for meat and milk up to 100 kg/ha. The present study has demonstrated that high antimicrobial concentration in soil is present, which can cause health problems.

To compare the influence of different sewage sludge composting methods to the degradation rate of the fluoroquinolones and sulfonamides in it, some new experiments were performed. The sewage sludge was mixed with different materials as tree bark, peat, oil shale ash, litter and sawdust. The antimicrobial decomposition rate dependence on added material was determined. Antimicrobials were extracted from compost samples using PLE, extracts were cleaned up by SPE and analyzed by LC-MS. The results will be presented. This work will be performed to optimize the sewage sludge composting technology to produce safe agricultural fertilizer.

MO 312

Prospects of mancasanthus x giganteus for the rhizomethod of PAH contaminated soils


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Phytoremediation of large area surface using crop cultivation appears as an attractive strategy to allow for long-term and economically viable site rehabilitation programs. The phytomining process tries to characterize the phytomining potential of a different species x giantazanthus x giganteus. For this purpose, three factors affecting bioremediation, namely the nature of soil contamination (spiked-phenanthrene and pyrene each at 100 mg/kg dry soil), bioaugmentation (with a PAH-degrading microflora) and planting (with Mancasanthus x giganteus), were arranged according to a full factorial design in a pot experiment. Their effects (alone or in combination) were assessed on soil quality (total and degrading microflora enumeration, 16S-rDNA molecular diversity analysis) and PAH dissipation. Results showed that contamination eluents with different organic modifiers and different buffers at a range of pH were tested. The results of these experiments, where the sorption mechanism was known, were compared to results obtained using ‘in house’ prepared cartridges of sludge at different sludge/silica ratios.
In the present study we will analyse levels of 26 PAHs and 12 oxy-PAH derivatives during a full scale biological remediation of a PAH contaminated soil using GC-MS. Our objective is to improve the knowledge of the changes on PAH and oxy-PAH levels during the remediation process (2) study the availability of the compounds over time using methanol extraction and (3) study if the toxicity in the soil is reduced in proportion to the putative reduction of the PAHs, using the H4IIE-luc dioxin bioassay, based on hepatocyte cell lines. Mass balance analysis will show how much of the AHr-mediated activities in the soil the GC-MS analysed compounds account for. The results will give information of the role of PAHs and oxy PAHs found in PAH contaminated soils, the degradability of these compounds, formation of oxy PAHs and changes in availability during a biological remediation.

### MO 315

**Ecotoxic problems of erosion, salinization and alkalization of soils in Azerbaijan**

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The exposure to soil at different level of soil erosion is one of the characteristic ecological problems of our Republic. But real disaster of land management in republic and in the world is clear, the over-abuse of chemical fertilizers, irrigation, deforestation, processes of soil erosion and desertification. Land and desertification process and intensively influences to erosion process and gradually changes under its influence. At the result of agricultural activities 30 th. ha of land cover destructed, but 40% of land cover exposed to erosion processes, result of wrong land reclamation work shows its negative influence, in results underground water level and salinization of arable regions in increase. In arable regions of the country more harm gives irrigational erosion. In appropriation of mountain mass under not follow anti erosional measures, the soil will decrease which shows negative influence on productivity and often cases take out from the plant from agricultural cultivation. Very often anti erosional processes causes washout of the 100 ha soil, especially in irrigation station. Mountain and plain regions of Azerbaijan exposed to irrigational erosion. At the result of wrong irrigation irrigation productivity of the land decreases. Research works shows that total lands of territory exposing to erosion is 31444,7 th. ha in our Republic, which means 16%, 31% and 25% of total area. Problem of salinization and alkalization are the most serious problems among the eco-geographical problems. Nowadays approximately 37000 th. ha soils of all existing land (8641560 ha) in Azerbaijan exposed to degradation. Soil salinization is widespread in Azerbaijan. 60% of Kur-Araz plain occupying 2,2 million ha territory consists of average and full saline soil. Generally in the territory of our Republic total area of average and full saline soil is 1.3 mln ha. That is why these lands with agricultural production are considered as on the edge of extinction and main reason of land desertification. Emission of the 16 priority PAHs, even though often hundreds of other PAHs and oxy PAHs are widely spread in Mexicali valley, with DDTs accounting for the highest contribution to the total pesticides, mainly the 4,4-DDE (50-80%). The highest concentration of DDTs in soil samples was found in the soil from the area of the town of Mexicali, where the highest concentration of DDTs in soil samples (up to 0.4 mg/kg) was found. The highest concentration of DDTs was found in the soil samples from the area of the town of Mexicali, where the highest concentration of DDTs in soil samples was found (up to 0.4 mg/kg).

### MO 316

**Ecological characterization of Mountain Shirvan regions of Azerbaijan**

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The composition of Mountain Shirvan economic and geographic region includes Agsu, Ismailli, Gobustan and Shamakha districts. Total area of the region is 613 thousand ha, or 27% of area of Azerbaijan. The region is divided into districts and subdistricts. On the plains territory the climate is hot and dry subtropical; on the upland places - the summer is cool, the winter is rather cold and arid. Annual middle temperature of foothill-plan territory is 14,10C (Agsu), in upland territory -10,50C. Temperature of the coldest month in Agsu is 3,30C, in Shamakha 0,60C. Annual rainfall is 1635mm in Agsu, 642mm in Maraza. Of total territory 53% of area is upland and 185 thousand hectare (30,2%) is foothill-plan. Composition of the land cover: 37,2%-mountain chestnut; 11,4%-chestnut; 11,8% brown and brown mountain-forest; 3,3%-black; 8,2%-grey; 8,0%-alluvial-meadow;2,7%-meadow-forest; 5,7%-mountain-forest. Composition of land supply: useful lands for agriculture - 377676 ha (67,1%), from arable them - 37,5% (141,3 thousand ha), pasture - 2,5% (9,2 thousands hectares), hayland-1,2% (4,6 thousands hectares), pastures -57,1% (312,5 thousands hectares). Garden plot for 2,5% (9191ha), forest - 13,3% (185596ha). Irrigated lands useful for agriculture is 52,8 thousand hectare (14,2%). From vegetation - cereal-growing, potato-growing, vegetable-growing, vine-growing, melon-growing, fruits-growing, cotton-growing, feeding-grow. In subalp meadows are meadows: moderate-damp-cereal, mix-cereal, damp- meadow, mountain meadow; 27,4% of lands are humidified, subalpine chibi and subalpine meadows 34% of lands. The Reletion of the Alps belt wide spread within 2400-3200 above s.l. is also expressed by meadow and carper elements. The vegetation at these heights is formed, mainly, on gentle slopes, dome-shaped tops, saddles. Data of the spent researches give representation about spatial laws of ecosystem distribution of Mountain Shirvan with different disturbs level and also data on change of typical structure of soils and specific structure of vegetation accordingly.

### MO 317

**Influence of anthropogenic factors on landscape complexes in mountain areas**

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In modern age which the society is developing rapidly and has great technical strength, the relation between nature and human begin to develop in a strong manner. Even in the mountain areas, which are more natural, the human impact is noticeable, and even were not found yet many ways of using natural resources with a great impact. Environmental protection, nature conservation, and sustainable development of natural resources are the main goals of current studies. The main objective of this study is to understand the influence of anthropogenic factors on landscape complexes and to provide recommendations for sustainable development of these regions. The study was conducted in the mountainous areas of Azerbaijan, specifically in the region of Agsu, Ismailli, Gobustan, and Shamakha. The study aimed to assess the impact of human activities on the landscape complexes and to identify the most vulnerable areas. The study used a combination of remote sensing and field surveys to collect data. The results showed that human activities, such as deforestation, land use changes, and infrastructure development, have significantly altered the landscape complexes. The most vulnerable areas were identified as those with high human impact and low biodiversity.

### MO 318

**Organochlorine pesticides in soils from the agricultural valley of Mexicali, Baja California, Mexico**

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The study of soils and pesticides is crucial for understanding the impact of agricultural activities on the environment. The agricultural valley of Mexicali is a region with high pesticide usage, particularly organochlorine pesticides (OCPs), due to the intensive cultivation of crops. The study aimed to determine the levels of OCPs in soils from this region and to assess the potential environmental and health impacts. Soil samples were collected from various locations in the agricultural valley of Mexicali, Baja California, Mexico. The study used GC-MS analysis to detect and quantify the levels of OCPs. The results showed that the most commonly detected compounds were DDTs, DDEs, DDD, and DDDs, with concentrations ranging from 0.1 to 1.0 mg/kg. The highest concentrations were found in soils from areas with intensive agricultural activities. The study also assessed the potential risks associated with these levels of OCPs. The findings indicated that the levels of OCPs in soils exceeded the threshold levels set by the US Environmental Protection Agency (EPA). This highlights the need for more stringent regulations and monitoring to ensure the safety of the agricultural activities in this region.

### MO 319

**Environmental fate of heavy metals at small arms firing ranges**

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The study of heavy metals in soil is important for understanding the environmental impact of small arms firing ranges. These ranges are known to be sources of heavy metal contamination due to the use of ammunition. The study aimed to assess the levels of heavy metals in soils from small arms firing ranges and to evaluate the potential risks to the environment and human health. Soil samples were collected from various locations near small arms firing ranges in Canada. The study used atomic absorption spectrometry to determine the levels of heavy metals. The results showed that the most commonly detected metals were lead (Pb), copper (Cu), zinc (Zn), and cadmium (Cd). The highest concentrations were found in soils near firing ranges, with Pb and Cu levels exceeding the threshold levels set by Canadian regulations. The study concluded that the use of small arms firing ranges poses a significant risk to the environment and human health, and that more stringent regulations and monitoring are needed to mitigate these risks.
Different soil texture, organic matter content, crops, and sewage sludge application rates were chosen. In each parcel, soil samples were measured for POPs. In the directive (PAH, NP+NPEs, PCDD/Fs, PCBs, LAS) and other emergent organic contaminants (PBDEs, PCNs, PFOS+PFOA) were measured. Also, controlled parcels with evaluation and model development. First, 200 SS samples from 125 wastewater treatment plants all around Spain were collected and the concentrations of the POPs proposed. The main objective of this work was to evaluate POPs fate in soils amended with sewage sludge. To accomplish this objective, the work was divided in two parts: samples collection and analysis, and model development.

Methods specifically adapted for PAHs estimation are hardly used. Different solvents and extraction techniques have been used to determine the total concentration of PAHs in BC. Soxhlet extraction with dichloromethane, methanol, and hexane with Soxhlet, liquefaction extraction, pressurized fluid extraction, etc. Such approaches were often based on certified analytical procedures (e.g. ISO 38 414 using cyclohexane, DIN EN 15527 using petroleum ether) originally established for other matrices such as soil, and may lead to negligible or low total concentrations of PAHs in BCs, or low recoveries of PAH internal standards. Hence, traditional methods may fail short to determine PAHs quantitatively. The aim of this study is therefore to provide an easy and valid method for the quantitative determination of PAHs in BCs to researchers, practitioners, and legislators.

Four BCs of different feedstocks were extracted with Soxhlet with two different extraction durations and with accelerated solvent extractor. Different solvents were used such as toluene, hexane, dichloromethane/acetone (1:1, v/v) and others and different cleanup steps were applied. Total concentrations were measured by gas chromatography-mass spectrometry (GC-MS).

The extraction results will be presented which include the best extraction solvent and technique, the optimal extraction duration, linearity, detection limits, method precision, sample representativeness and absolute and relative recoveries. Some implications concerning the amendment of BCs will be critically evaluated.

We have developed a simple, robust and sensitive extraction method to quantitatively determine total concentrations of PAHs in BCs. This method may serve researchers, practitioners and legislators to optimize BC production with a view to minimize its PAH content, and to properly assess the environmental benefits and risks of this overall promising material.

Alteration of the reproductive toxicity of Cadmium in the soil living Nematode C.elegans upon potential chemosensitization by HHCB

M. Silva

Tetrodotoxin expansion in the temperate waters of the Atlantic Ocean new vectors and impact on marine ecosystems

I. Llorens, R. Yensen

Hydroxylated and methoxylated brominated diphenyl ethers: natural products or Anthropogenic Contaminants?

H. Schmidt, M. Heiniger, H. Frimmel, N. Hutzinger

Quantitative determination of PAHs in biochar - a prerequisite for its quality and safe application

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Biochar (BC) is pyrolyzed organic feedstock. It has become more and more important as soil conditioner and received broader attention as a possible measure against climate change. Polycyclic aromatic hydrocarbons (PAHs) is produced during BC production due to incomplete combustion. This work will enter the environment when the BC is applied as soil conditioner to avoid leaching of nutrients, to increase microbial biomass and activity, to remediate the soil of organic pollutants, etc. Such positive effects are undermined if PAH exposure is concomitantly increased.

Methods specifically adapted for PAHs estimation are hardly used. Different solvents and extraction techniques have been used to determine the total concentration of PAHs in BC. Soxhlet extraction with dichloromethane, methanol, and hexane with Soxhlet, liquefaction extraction, pressurized fluid extraction, etc. Such approaches were often based on certified analytical procedures (e.g. ISO 38 414 using cyclohexane, DIN EN 15527 using petroleum ether) originally established for other matrices such as soil, and may lead to negligible or low total concentrations of PAHs in BCs, or low recoveries of PAH internal standards. Hence, traditional methods may fail short to determine PAHs quantitatively. The aim of this study is therefore to provide an easy and valid method for the quantitative determination of PAHs in BCs to researchers, practitioners, and legislators.

Four BCs of different feedstocks were extracted with Soxhlet with two different extraction durations and with accelerated solvent extractor. Different solvents were used such as toluene, hexane, dichloromethane/acetone (1:1, v/v) and others and different cleanup steps were applied. Total concentrations were measured by gas chromatography-mass spectrometry (GC-MS).

The extraction results will be presented which include the best extraction solvent and technique, the optimal extraction duration, linearity, detection limits, method precision, sample representativeness and absolute and relative recoveries. Some implications concerning the amendment of BCs will be critically evaluated.

We have developed a simple, robust and sensitive extraction method to quantitatively determine total concentrations of PAHs in BCs. This method may serve researchers, practitioners and legislators to optimize BC production with a view to minimize its PAH content, and to properly assess the environmental benefits and risks of this overall promising material.
Recently published data showed that the aquatic environment can also be exposed to mycotoxins. The identified main input sources of mycotoxins into the aquatic environment were discussed and help us to differentiate and understand the anthropogenic and natural sources of hydroxylated and methoxylated BDEs found in the marine environment.

Mass balance of 81Br in the algal and water samples was constructed to test the hypothesis that OH-/MeO-BDEs are synthesized by the algae. Result of this study will be used to help us understand the anthropogenic and natural sources of hydroxylated and methoxylated BDEs found in the marine environment.

MO 328

Assessing the toxicity of two natural bioactive compounds applied in aquaculture using zebrafish embryos

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In this study, four species of plankton algae (two diatom species: Thalassiosira pseudonana and Skeletonema costatum; and two dinoflagellate species Prorocentrum dentatum and Prorocentrum minimum) were cultured in (a) Br free artificial sea water and (b) artificial seawater with isotope 81Br under laboratory conditions. Levels of 9 common MeO-BDEs analogues, 10 OH-BDEs analogues and 12 Brominated Phenol (BRPs) analogues were determined by GC-MS.

Environmental responsibility, nematode management and collaboration between academia and industry

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MO 333
The effects of oral administration of toxic cyanobacteria on multiple physiological, biochemical, hematological and immunological parameters in rats

Cyanobacteria are known producers of wide spectrum of compounds with various effects such as skin irritation, gastrointestinal tract and respiratory distress, neurotoxicity, immunotoxicity or hepatotoxic, cancerogenic, genotoxic and mutagenic effects. Recent studies have shown also endocrine disruptive potential of specific cyanobacterial and algae could contribute to endocrine disruptive potential in surface waters. Aim of our study was to characterize impact of external factors such as light, and variability of production of endocrine disruptive compounds and microcystin during growth phase. The detailed characterization of the cultures enabled to quantify the concentration of up to 65 ng/g lipids. The discovery that halogenated phenols, at least in mussels, can occur as lipid soluble neutral conjugates in high amounts, under natural conditions, is new. The repercupussions of these findings may be that conventional analyses to assess the total amount of halogenated phenols, in e.g. mussels, have underestimated the true levels.

The present study was undertaken to investigate the possible conjugation of OH-PBDEs, with lipophilic substrates (e.g. fatty acids), in mussels under natural conditions. Such conjugates could have environmental significance as they are produced in the potential to act as endocrine disruptors. The research was supported by the Czech National Science Foundation grant No. 524/08/0496 and by the project DOP which showed the highest mean concentration. Other compounds detected occasionally included beauvericin, and 3-acetyl-deoxynivalenol. Different approaches to the investigation period, four mycotoxins were detected regularly, with nivalenol being the most prominent mycotoxin in terms of frequency of occurrence, closely followed by DON which contained up to 65 ng/g lipids.
Experimental rats were fed for 28 days with food containing fish meat with external additions of isolated microcystins, biomass of toxic cyanobacteria Microcystis, nontoxic cyanobacteria Arthrospira and also green alga Chlorella. Subgroups of the animals were also challenged with a model antigen KLH to investigated immune-related parameters. Although considerable amounts of microcystins were orally administered to rats, levels of MCs in the tissues were below the detection limit (1 ng/g fresh weight; based on the tandem LC/MS). Only traces of the conjugates of microcystins with cystein and glutatimation were detected in the rat's liver exposed to Microcystis biomass. The feed containing cyanobacterial biomass was found to have negative impacts on the weight gain and food conversion ratio of experimental rats. Interestingly, rats exposed to MCs had stimulated immune system (higher antibody responses to administered KLH antigen). Also modulations of several hematological parameters of blood were significantly modulated. Our study demonstrates that oral exposure to microcystins or cyanobacterial biomass may have different impacts on various less explored biochemical and immune parameters in experimental mammals.

MO 340

Protective role of N-Acetylcysteine against the oxidative damage induced by cylindrospermopsin in tilapia (Oreochromis niloticus)

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Cylindrospermopsin (CYN) is a cytotoxic cyanotoxin produced by several species of cyanobacteria. CYN consists of a tricyclic alkaloid with a high solubility in water, where it can be present approximately the 90% of the total CYN produced by cyanobacteria. Aquatic animals and plants can be exposed to CYN, and it is known that CYN is a potent inhibitor of protein synthesis and reduced glutathione (GSH). Moreover, several recent studies have established that CYN produces oxidative damage through the generation of reactive oxygen species (ROS).

As the presence of CYN in freshwater systems could have negative effects on fish it would be interesting to find compounds able to counteract the toxicity observed. In this study, the protective role of N-acetylcysteine (NAC) as a GSH precursor which can protect organs against the oxidative damage through two different mechanisms: by the stimulation of the GSH synthesis and by direct association to ROS. In the present work, we aimed to investigate the effects of pure CYN and CYN from a lyophilized Aphanizomenon ovalisporum culture on fish, and the protective role of a pre-treatment (7 days) with different doses of NAC. Both products (CYN and NAC) were administered together with the food. Fish were euthanized after 24 hours of the toxin exposure, and liver and kidney were extracted. For this purpose, we used different oxidative stress biomarkers such as lipid peroxidation, γ-glutamylcyclsteine synthetase (GCS) activity, and GSH levels. Results showed that CYN induced oxidative stress, as evidenced by the increase of lipid peroxidation, the decrease in GSH/GSSG, and the alteration of the GCS activity. Moreover, the exposure to cyanobacterial cells containing CYN induced higher toxic effects in comparison to pure CYN. NAC supplementation was effective in reducing the CYN induced toxicity, particularly at the highest dose employed (45 mg/fish/day). In conclusion, NAC can be considered a useful chemoprotectant in the prophylaxis of CYN-related oxidative stress induction in fish.

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MO 341

Preliminary study of biomarkers in mussel haemolymph after exposition to toxins extracted from Microcystis aeruginosa and Lyngbya wollei

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The freshwater cyanobacterium Lyngbya wollei is a dominant primary producer in many lakes and reservoir, whereas Microcystis aeruginosa can become a dominant cyanobacteria commonly associated with eutrophic conditions. Both cyanobacteria produced toxin, M. aeruginosa (MCYST) produced a potent specific hepatotoxic, whereas L. wollei (LYNGTX) produced an analogue to saxitoxin which is a neurotoxin. Selected immune parameters investigating haemolymph integrity and immunocompetence after exposure of the two toxins by flow cytometry of non-cytotoxic low concentrations of MCYST and LYNGTX. Exposure of freshly isolated mussel haemolymph to low concentrations of 2, 5, 10, 25, 50ng/ml for 18h periods resulted in a significant interaction term (concentration x type of toxins) of the two-ways ANOVA of haemocyte viability. Haemocytes exposed to MCYST tended to decrease their viability at the highest concentration whereas haemocytes exposed to LYNGTX showed a positive relationship with their viability at the highest concentration. However, the type of toxin was significantly different for phagocytosis activity. Haemocytes exposed to MCYST had a lower phagocytic activity than haemocytes exposed to LYNGTX. Furthermore, the present study will investigate the effects of these toxins on the intracellular thiols production, reactive oxygen species (ROS) production, cyclo-oxygenase activity (COX) and nitrate production in mussel haemolymph. Preliminary results showed that haemocytes exposed to MCYST showed a higher expression of the gene encoding the lipoxygenase. A decrease of the phagocytic index is usually observed response to in vivo or in vitro exposures to toxic concentrations of xenobiotics where MCYST seems to have a similar effect. This preliminary study will require additional biomarkers to support the hypothesis of a toxic effect associated with the mats of filamentous L. wollei.

MO 342

Phosphatases and Esterases of Limnoperna fortunei (golden mussel) in presence of Microcystis aeruginosa

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The freshwater cyanobacterium Microcystis aeruginosa is commonly associated with eutrophic conditions. Both cyanobacteria produced toxin, M. aeruginosa (MCYST) produced a potent specific hepatotoxic, while combinations of tebufenpyrad and Cylindrospermopsis demonstrated antagonistic effects on reproduction of Daphnia. Results suggest that MCYST can become a dominant cyanobacteria commonly associated with eutrophic conditions. Both cyanobacteria produced toxin, M. aeruginosa (MCYST) produced a potent specific hepatotoxic, whereas L. wollei (LYNGTX) produced an analogue to saxitoxin which is a neurotoxin. Selected immune parameters investigating haemolymph integrity and immunocompetence after exposure of the two toxins by flow cytometry of non-cytotoxic low concentrations of MCYST and LYNGTX. Exposure of freshly isolated mussel haemolymph to low concentrations of 2, 5, 10, 25, 50ng/ml for 18h periods resulted in a significant interaction term (concentration x type of toxins) of the two-ways ANOVA of haemocyte viability. Haemocytes exposed to MCYST tended to decrease their viability at the highest concentration whereas haemocytes exposed to LYNGTX showed a positive relationship with their viability at the highest concentration. However, the type of toxin was significantly different for phagocytosis activity. Haemocytes exposed to MCYST had a lower phagocytic activity than haemocytes exposed to LYNGTX.

MO 343

Do mixture effects of metal stress (Cu) and natural stress (cyanobacterial toxins) add up in Daphnia magna?

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Current risk assessment of chemicals does not deal with mixture toxicity, which contrasts with ecology reality. The combined effect of stressors is not necessarily bound to the generic pattern of additivity but can instead result in their combined effect being either more or less toxic than would be expected from their individual toxicity (synergy or antagonism).

We investigated the combined and interactive effects of dissolved copper (Cu) and cyanobacteria in two Daphnia magna clones (Xinb3 and Xinb1). Several cyanobacterial species (Microcystis aeruginosa, Cylindrospermopsis raciborskii, Anabaena sp., Nodularia sp., Oscillatoria sp., Aphanizomenon fluosace) were investigated, ensuring toxins with different Modes of Action (MoA). Semi-static 21-day life-table experiments were performed according to an extended second-order two factor central composite design, considering all possible combinations of toxins and life-history stages. In each combination, two control groups were used (reference and the reference added to a high concentration of one of the xenobiotics). The concentration of the different combinations was calculated on the basis of a 0-1 modulation by the ratio of the effective size of each compound to the size of the fish, here defined as the fish size at the end of the experiment. The treatment was performed as a mixture of the two compounds with different dosages and ratios. Results were analysed with the mixtox model developed by Juner et al. (2003 Environ Toxicol Chem 24:2701-2713). The fittted model can be used to make predictions on the magnitude of increase or decrease of Cu toxicity in the presence of cyanobacteria.

MO 344

Interactive effects of combined chemical and natural stressors on Daphnia pulex

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Current risk assessment of chemicals does not deal with mixture toxicity, which contrasts with ecology reality. The combined effect of stressors is not necessarily bound to the generic pattern of additivity but can instead result in their combined effect being either more or less toxic than would be expected from their individual toxicity (synergy or antagonism).

We investigated the combined and interactive effects of copper (Cu) and cyanobacteria in two Daphnia magna clones (Xinb3 and Xinb1). Several cyanobacterial species (Microcystis aeruginosa, Cylindrospermopsis raciborskii, Anabaena sp., Nodularia sp., Oscillatoria sp., Aphanizomenon fluosace) were investigated, ensuring toxins with different Modes of Action (MoA). Semi-static 21-day life-table experiments were performed according to an extended second-order two factor central composite design, considering all possible combinations of toxins and life-history stages. In each combination, two control groups were used (reference and the reference added to a high concentration of one of the xenobiotics). The concentration of the different combinations was calculated on the basis of a 0-1 modulation by the ratio of the effective size of each compound to the size of the fish, here defined as the fish size at the end of the experiment. The treatment was performed as a mixture of the two compounds with different dosages and ratios. Results were analysed with the mixtox model developed by Juner et al. (2003 Environ Toxicol Chem 24:2701-2713). The significance of model deviations for synergistic and antagonistic effects were determined through maximum likelihood, while regression analysis was performed to select the best reference organism. The analysis resulted among others in additive effects in combinations of carbaryl with Cylindrospermopsis, while combinations of tebufenpyrad and Cylindrospermopsis demonstrated antagonistic effects on reproduction of Daphnia. Results suggest complicated interaction patterns between insecticides and harmful cyanobacteria potentially related to mode of action patterns.

MO 345

Physiological effects of a marine algal toxin on a primary consumer: tales of the unexpected

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Waterblooms of cyanobacteria (blue-green algae) can produce potent toxins that have become a severe problem for eutrophic aquatic environments. Hepatotoxins are potent entities that can cause serious health risks and economical losses due to the production of toxins. Okadaic acid (OA), a marine toxin produced by different species of dinoflagellate, accumulates in filter-feeding shellfish, OA is known to inhibit the protein phosphatase enzymes in humans consuming contaminated shellfish, resulting in the diarrheic shellfish poisoning syndrome.

Although the physiological effects of OA on mammals are relatively well understood, this is not the case for the effects on the main vector (i.e. shellfish). In this study, the effects of a novel non-specific (NS) membrane stability (LMS) of the hepatocytes and on the protein phosphatase (PPase) activity of the hepatopancreas in the mussel Mytilus edulis were studied. In the first phase the mussels were dissected and the enzymes of the hepatopancreas were exposed to okadaic acid in vitro (conc. up to 672 μg OA/g hepatopancreas WW). This resulted in a significant reduction (4%) of the PPase activity, starting at a concentration of 26.2 ng OA/g hepatopancreas WW, with a maximum inhibition of approximately 15% at 168 ng hepatopancreas WW and higher. In the second phase, the mussels were exposed in vivo to toxin producing algae (P. lima).

Two concentrations were tested: 2.85 μg OA/L (500 cell/ml) and 9.55 μg OA/L (1500 cell/ml). Okadaic acid accumulated in the mussels’ hepatopancreas with a mean concentration of 2.20 ng/gWW (500 cell/ml) and 740 ng/gWW (1500 cell/ml). Based on the in vitro results a 15% reduction in PPase activity was expected. However, there was no significant reduction of the LMS or the PPase activity.

Two conclusions can be drawn from these results. First, in vivo there is a reduction of the PPase activity this means that these enzymes are sensitive to OA exposure. Secondly, in vivo OA has no negative effect on the PPase activity or LMS. Apparently M. edulis is, at the concentrations tested, able to prevent the inhibition of protein phosphatase enzymes by OA.

MO 346

Oxidative stress responses produced in liver of tilapia by exposure to repeated doses of cylindropermopsin from Aphanizomenon ovalisporum R. Guzmán-Guillén1, A.I. Prieto1, C. Fernández-Blanco1, V.M. Vasconcelos2, A.M. Caneán1

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Cylindropermopsis (CYN) is a triacyl lipid, possessing a triacyl guanidine moiety combined with hydroxymyristic acid. It is produced by several genera of cyanobacteria, growing worldwide in eutrophic freshwaters and it is easily transferred among different trophic levels causing damages to humans, animals and plants. It is well established its mechanism to block protein synthesis, and more recently, its ability to cause oxidative stress is being studied.

The aim of this work was to study the oxidative stress responses in liver of tilapia (Oreochromis niloticus) exposed to both a culture and lyophilized cells of Aphanizomenon ovalisporum (CLEGE X-001). These parameters were: lipid and protein oxidation, and the enzymatic activities of Glutathione-S-transferase (GST), Glutathione Peroxidase (GPx), Catalase (CAT) and Superoxide dismutase (SOD).

In this study, nine groups of tilapia with 8 fish per group were established: 3 control groups, 3 groups exposed by immersion to an A. ovalisporum culture (10 μg CYN/L), and Oscillatoriales in pre-reservoirs. In 2007, Oscillatoriales, dominated by Planktothrix agardhii, largely prevailed in deep water whereas Nostocales were more present in pre-reservoirs; Chrococcales samples. In 2010, 60% of the samples were positive for at least one of these toxins. Nevertheless, total concentrations remained low in the freshwater samples analysed (<< 1µg/L).

Two conclusions can be drawn from these results. First, in vivo there is a reduction of the PPase activity this means that these enzymes are sensitive to OA exposure. Secondly, in vivo OA has no negative effect on the PPase activity or LMS. Apparently M. edulis is, at the concentrations tested, able to prevent the inhibition of protein phosphatase enzymes by OA.

MO 347

Development of a method for determination of CYN in lyophilized Aphanizomenon ovalisporum cells by LC/MS/MS: validation and application to real samples

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Microcystins are the most intensively studied toxins and numerous reports on their occurrence exist all over the world. A WHO guideline value of 1 μg/L Microcystin-LR exists for drinking water, which has been adopted in several European guidelines (e.g. France). In contrast, little is known about the distribution of other hepatotoxins such as cylindropermopsin (CYN), prorocentrin (PRT) and nodularin (NOD) in water. Therefore it is important to develop and validate a method to determine CYN at trace levels. The aim of this work was to develop an analytical procedure based on solvent extraction followed by a purification step involving on- and off-line SPE chromatography and LC/MS/MS for CYN determination from lyophilized cultures of A. ovalisporum (CLEGE X-001). The extraction and purification steps were optimized using a two-level full factorial design with replications.

This report presents a sensitive, reproducible, accurate, and robust method for extraction and determination of CYN in lyophilized cells. The recoveries (83-94%) and intermediate precision values obtained (5.6-19.3 %), as well as the robustness of the method for the three factors considered, permit its validation. This method provides acceptable detection and quantitation limits for environmental studies and proves its utility for monitoring CYN in lyophilized natural blooms samples, and also for routine human health assessment purposes in relation to the provisional guideline and TD, representing an available instrument to advance in these studies.

Acknowledgements

The authors wish to thank the Spanish Ministerio de Ciencia e Innovación (CICYT, AGL2009-10026ALI) and Junta de Andalucía (P09-AGR-4672) for the financial support for this study.

MO 348

Screening for cylindropermopsin, anatoxin-a and saxitoxin in France

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Cylindropermopsin and saxitoxin can be produced in natural waters, leading to harmful algal blooms. Harmful algal blooms are occurring in eutrophic freshwater lakes and reservoirs throughout the world and can present a public safety hazard through contamination of drinking water supplies. There are over 40 species of cyanobacteria representing 20 genera from three cyanobacterial families which include both cyclic peptides and alkaloids such as cylindropermopsin (CYN), produced by Aphanizomenon ovalisporum among other species. Therefore it is important to develop an analytical method to determine CYN in trace levels. The aim of this work was to develop an analytical procedure based on solvent extraction followed by a purification step involving on- and off-line SPE chromatography and LC/MS/MS for CYN determination from lyophilized cultures of A. ovalisporum (CLEGE X-001). The extraction and purification steps were optimized using a two-level full factorial design with replications.

This work proposes a sensitive, reproducible, accurate, and robust method for extraction and determination of CYN in lyophilized cells. The recoveries (83-94%) and intermediate precision values obtained (5.6-19.3 %), as well as the robustness of the method for the three factors considered, permit its validation. This method provides acceptable detection and quantitation limits for environmental studies and proves its utility for monitoring CYN in lyophilized natural blooms samples, and also for routine human health assessment purposes in relation to the provisional guideline and TD, representing an available instrument to advance in these studies.

Acknowledgements

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MO 349

Sensitive detection of microcystins using online SPE from environmental waters

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Waterblooms of cyanobacteria (blue-green algae) can produce potent toxins that have become a severe problem for eutrophic aquatic environments. Hepatotoxins are among the toxins produced by these species growing in lakes, ponds, and rivers used as drinking water sources. Microcystin contamination of drinking water at low nanomolar concentrations is considered a risk factor for cancer, and microcystin-LR has been associated with most of the incidents of toxicity involving microcystins. Therefore, the World Health Organization (WHO) has proposed a provisional guideline concentration of 1.0 μg/L for microcystin-LR in drinking water. The authors have reported a simple, fast, and effective target-cutoff on-line SPE method followed by HPLC with UV detection. This on-line SPE method is different from the typical one. The bound analyte on the SPE column is selectively eluted from the SPE column using a mobile phase gradient, just like the first dimension of a two-dimensional chromatography system. This reduces the number of interferences for sample analysis. Here, the target-cutoff on-line SPE method followed by HPLC with UV detection was applied to the determination of three microcystins (L-, RR-, and YR) in drinking water. The three target analytes were co-eluted from the first column using chromatographic conditions that eliminated as many interferences as possible; then the analytes were sent to the analytical flow path and separated on the second column using the same type of stationary phase under different chromatographic conditions. This design takes advantage of the separation power of both columns and may eliminate interferences more efficiently than typical on- and off-line SPE methods. Sub-ng/L concentrations of microcystins-LR, RR- and YR spiked in water samples were determined, which exceeds the WHO requirement.
Hazardous formation of microcystins by filamentous cyanobacteria in urban lakes using molecular and biological methods. A case study in Mexico City
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MO 350

Hazardous formation of cyanobacteria blooms in water bodies with a high nutrient content (mostly P and N) is a frequent occurrence affecting freshwater quality at all latitudes. Bloom development is of great concern since cyanobacteria can biosynthesize a large number of secondary metabolites, some of which are toxic to aquatic biota and mammals, including humans.

MCs are potent inhibitors of the protein phosphatase 1 and 2A. Zooplankton filter-feeders such as cladocers are directly affected by MCs as a result of ingestion of microcystins or toxic cyanobacteria. This study evaluates the potential toxicity of microcystins produced by cyanobacteria in urban lakes of Mexico City.

The study was carried out in 17 urban lakes across Mexico City during 2014. Water samples were collected every 3 days for cell counting, chlorophyll a (chl-a), cyanobacteria and microcystins analysis. Analytical methods used in the present study (PCR and ELISA) were useful and reliable for determining MC production and its potential contribution to the acute effects observed in D. magna. The results indicated that blooms of MC-producing cyanobacteria are frequent in these urban water bodies.

The presence of cyanobacteria can be a threat to aquatic biota and mammals, including humans, due to the production of secondary metabolites, such as microcystins. These compounds have been associated with a variety of toxic effects on aquatic organisms, such as inhibition of protein phosphatases, cytotoxicity, and neurotoxicity.

The study highlights the importance of monitoring cyanobacteria blooms in urban lakes, as they can pose a significant risk to human and animal populations. Further research is needed to better understand the factors that influence cyanobacteria blooms and the toxicity of microcystins produced by these organisms.
Swiss mice blood. Group of 9 or 10 animals were treated intraperitoneally (ip) with 20 mg.kg-1 of antx-a(s)-containing extract and sacrificed after 24 h, 48 h, 7 and 14 days of treatment by cardiac puncture.

The ACHE and BuCHE activities in mice treated with antx-a(s)-containing extract stayed inhibited more than 55% during 48 h (n=9, p<0.001). Normal activity of both enzymes was observed after seven days of treatment. By the other hand, changes in the antioxidant enzymes activities only began after the second day of treatment. Initially, both CAT and GPX showed lower activity than the control group after 48 h. Among those enzymes, GPX showed the highest decreased activity (n=9, p<0.001). After seven days, while the antx-a(s)-containing extract promoted increasing of CAT and GR activities, GPX activity remained deeply decreased (n=10, p<0.01). SOD activity did not show significant difference related to the control during all the treatments. Activity of all the evaluated enzymes was completely recovered after fourteen days. From the obtained results, it can be concluded that the antx-a(s) extract demonstrated its pro-oxidant capacity in mice at sub-lethal levels. This fact was proved through the imbalance on the activity of the antioxidant enzymatic defense system.

**MO 358**

Physiological effects of phytotoxins in plants - laboratory studies to evaluate the impact of toxic cyanobacteria in agriculture, food quality and human health
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Toxic cyanobacteria outbreaks are recognized as emerging environmental threats worldwide. The process occurs in inland water systems as in some coastal areas and is becoming widespread mostly as a consequence of human activity, leading to nutrient input increases and degradation of water quality. The cyclic heptapeptide microcystin (MC) is produced by cyanobacteria that bloom in freshwater. This toxin has been directly linked to cases of human poisoning and to a prevalence of cancer induction in populations with chronic exposure to MC. Cylindrospermopsin (CYN) is another secondary metabolite produced by cyanobacteria. It is a tricyclic alkaloid with bioactivity. These compounds have shown to produce effects in a variety of aquatic organisms, vertebrates and mammals. Reports regarding variations in the animal behavior, growth, and mortality have led to the stress induced by cyanobacteria on the environment and animal health.

Recent investigation supports the hypothesis of accumulation of MC and CYN in plant tissues. Nevertheless a major gap lies in the physiological response of plants to phytotoxins and the mechanisms of uptake and translocation of these molecules from roots to shoot. This knowledge is essential to evaluate the impact of the use of contaminated waters in agriculture, food quality and human health. In this presentation we will discuss preliminary findings regarding the interaction of Lycopersicum esculentum and Oriza sativa plants with MC and CYN. Hydroponic cultures have been established and plants exposed to either isolated toxins or cyanobacterial cell extracts with concentrations varying between 1 µg/L and 300 µg/L. Short term exposures to both toxins does not alter the quantum yield of PSII in O. sativa and in L. esculentum after 15 days of exposure or the growth rate of plants. Nevertheless proteomic analyses displayed variations in protein expression in L. esculentum leaves suggesting a biochemical response of plants. Analysis of the activity of antioxidant enzymes is being undertaken to estimate the oxidative stress in plants in response to the phytotoxins.

Moreover HPLC and mass spectrometry techniques are being applied to quantify MC and CYN in plant tissues. We expect with this additional data to gather new insights in the toxicity of phytotoxins in these plant species and in the role of bioaccumulation in food quality and human health safety.

**ET16P - Quantitative Structure Activity Relationship (QSARs) and similar models for predicting the toxicity of chemicals, mixtures and combined stress**

**MO 361**

Environmental impact analysis of 10 active pharmaceutical ingredients with QSARs
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The environmental risk assessment of an active pharmaceutical ingredient (API) follows a stepwise approach, with a base set of studies on aquatic toxicity and fate as a start. This base set can be generated in standard tests, but at first the potential effects on aquatic organisms and the environment can be calculated to screen for possible alerts. Quantitative Structure Activity relationships (QSARs) are mathematical methods for estimating intrinsic environmental (hazardous) properties of chemicals, while reducing time, monetary cost and animal testing.

In this study QSAR models are used to calculate physical-chemical properties, acute and chronic ecotoxicity and environmental fate of 10 APIs from multiple therapeutic categories.

Calculations were performed in EPISuite. Model estimations are discussed and compared with measured data.

QSARs show to be a reliable tool for screening environmental properties of APIs. All the test result on (non) ready biodegradability were confirmed by the predictions of the Biowin model. All the ecotoxic substances were identified by Ecosar. The acute toxicity is in line with measured values (74%) or overestimated (26%) by the model. For the chronic toxicity, the lowest chronic value determines the PNEC and hence the outcome of the risk characterisation. The calculated lowest chronic value for each API in line with measured or lower. For the Ecosar classes considered for these 10 APIs, the main parameter in the algorithm is the log Kow which is inversely related to the ecotoxicity value. For the APIs with only covariant bonds the results with measured and calculated ecotoxicity are in the same order of magnitude. It can be argued for the salt toxicity that previously used the measured log Kow in order to refine the estimation and avoid underestimation of the ecotoxicity by the model. However further research is needed on this subject.

As part of an intelligent testing strategy (ITS) within risk assessment, QSAR predictions can deliver added value to other non-test methods such as in vitro tests, read across, grouping and weight of evidence approach, in order to minimise animal testing by focusing on those endpoints that may be of concern and to secure a high level of safety for man and the environment.

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**MO 362**

Extrapolative predictive QSAR models: thresholds of acceptance by various external validation criteria and critical inspection of scatter plots
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The evaluation of linear regression QSAR models performances, both in fitting and external prediction, is of pivotal importance. In the last decade different external validation parameters have been proposed: $Q_{ref}^2$ (Shib, $Q_{ref}^2$ (Scherumman), $Q_{ref}^2$ (Ledesma), averaged $r^2$ (Roy) and the Golbraikh & Tropsha (GT) method. Recently, the concordance correlation coefficient (CCC) has been proposed by our group as an external validation parameter to be used in QSAR studies. In our recent work, published in 2011 on JICIM, we have shown that, contrary to the commonly used acceptance thresholds ($Q_{ref}^2 > 0.6$, averaged $r^2 > 0.5$), the concordance correlation coefficient threshold value (0.85) is usually the most restrictive in the acceptance of QSAR models as externally predictive. This fact suggested that the CCC could be used as the main parameter in a precautionary approach, if the aim of QSAR developers is to have the smallest differences among the experimental data and the predictions of the external data set.

In this new work, we have studied and compared the general trends of the various criteria in dependence of different possible bias in the external data distributions (scale and location shifts), by means of a wide range of different simulated scenarios. This study highlighted, also by visual inspections of the experimental vs. predicted plots, some problems related to a few criteria; in particular, averaged $r^2$ values, if corrected for the model cutoff, could be prone to accept also not predictive models. This analysis allowed also to propose recalibrated, and inter-comparable, new thresholds for each criteria in the definition of a QSAR model as externally predictive. Two additional relevant topics emerged from the analysis of the results: 1) the scatter plot of the external predictions must always be evaluated and 2) the root mean squared error (RMSE) must also be calculated, as it is usually done in good QSAR practice. In fact, we have verified that the sensitivity of the various validation criteria to RMSE often differs. An additional important topic, here considered and applicable only to CCC, was to check by hypothesis test if the value of the calculated CCC is statistically significant. This procedure allowed, consequently, to determine also the minimum acceptable size of the external data set, an important point in QSAR studies, where the data set sizes are often small.

**MO 363**

Collection screening of QSAR models for REACH
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**REACH is the Regulation on Registration, Authorization, and Restriction of Chemicals. This legislation promotes the use of non-testing methods (NTM), which are all the approaches used to predict the effects of chemical compounds without the use of the real chemical compound. These methods include Quantitative Structure-Activity Relationship (QSAR) models and read-across. Before making an animal experiment the industry should verify if alternative methods exist. However, so far there is a deep gap of knowledge on which methods are available and can be used in practice.**

In this context a LIFEP project, named ANTARES, aims to reduce this gap assessing NTM as an alternative approach for the REACH legislation.

Here we report all validation studies carried out at IFR. In- house implemented QSAR models, both commercial or freely available through the internet. Almost 40 REACH endpoints are in principle covered by 250 QSAR models, 70 of them freely available. The full list of the QSAR models is available on http://www.antares-life.eu/index.php?sec=modellist.

The QSAR models cover endpoints for physico-chemical, environmental, ecotoxicological and toxicological properties. Within ANTARES we are evaluating in details the model development and evaluation process for different endpoints. Some examples of endpoints: biodegradation in bacteria; Acute toxicity by oral route; Short-term toxicity testing on invertebrates/Daphnia; Short-term toxicity testing on fish; Ready biodegradability; Bioconcentration factor. In this way we cover all the areas of the effects, giving preference to endpoints where more models and data exist. Within ANTARES we are evaluating the performances of these models with external test sets using specific criteria. Results will be discussed.

Moreover HPLC and mass spectrometry techniques are being applied to quantify MC and CYN in plant tissues. We expect with this additional data to gather new insights in the toxicity of phytotoxins in these plant species and in the role of bioaccumulation in food quality and human health safety.

**Acknowledgement** - The authors thank Janssen Pharmaceutica (Beerse, Belgium) of Johnson & Johnson for sponsoring this project

**Financial support from ANTARES project (LIFE 08 ENV/IT/000435) is gratefully acknowledged.**
QSAR classification models of the terrestrial toxicity of benzene triazoles: predictive tools for hazard and risk assessment

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Triazoles and benzotriazoles (TAZ/BTAZ) are potentially hazardous chemicals that adversely affect humans and other non-target species, and are on the list of substances of very high concern (SVHC) in the European regulation of chemicals REACH. TAZ/BTAZ are synthetic molecules, widely used in various industrial processes, as well as deicing agents, pharmaceuticals and pesticides. Because of their wide use they have been found distributed throughout the environment. The amount of experimental data available for these molecules is insufficient for a comprehensive characterization of their environmental and toxicological profile and they have been included among the four classes of chemicals studied in the European FP7 Project CADASTER (Case studies on the Development and Application of in-Silico Techniques for Environmental hazard and Risk assessment).

In this study quantitative structure-activity relationships (Q SAR) were used to model different endpoints of interest to define the potential toxicological profile of over 50 TAZ/BTAZ, with experimental acute toxicity data available for three key organisms of the terrestrial ecosystem. The studied end-points were: Honeybees 48 hour LD₅₀, Earthworm 14 day LC₅₀, Bird oral LD₅₀.

Different molecular descriptors were calculated by different proprietary and freely available online software (DRAGON 5.5 and PADEL-Descriptor 2.6). The endpoints of interest were modelled by classification (k-Nearest Neighbors (k-NNI)) and the best modeling variables were selected by Genetic Algorithm. The predictivity of the best models was validated by calculating the parameters sensitivity (Sn), specificity (Sp) and the non error rate (NER). External validation was also performed, depending on the availability of experimental data. The proposed models were submitted to the proposed procedure and the reliability of the predictions was always checked by the leverage approach in order to verify the chemical applicability domain of the models.

In conclusion, the proposed models, which were developed in R, could fill the data gaps and to support the activity of regulators with additional information to be used in terrestrial Hazard and Risk Assessment procedures.
respective, for MLR-OLS models, 0.93 - 0.99, 0.79 - 0.88 for PLSR models. The structural applicability domain to TAIZ/BTAZ in ECHA pre-registration list and the comparison of some models with ECOSAR predictions was also performed for the Insulins models.

The external predictivity of the models was compared by analysis of the Root Mean Squared Error (RMSE) and of other statistical parameters such as different external Q2 of the validation Coefficient Correlation (CCCV) recently proposed, by the Insulins group, for its use in QSAR validation.

**MO 369**

Assessment of alternative approaches to hazard identification of substances  
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The REACH Regulation stipulates that the principles of replacement, reduction and refinement of the use of animals in procedures should be fully taken into account in the design of the test methods, in particular when appropriate validated methods become available. REACH additionally promotes the use of (Q)SARs ((Quantitative) Structure Activity Relationships) because they contribute to the reduction of tests with vertebrates. The concept and use of alternative testing and (Q)SARs are therefore strongly encouraged in the REACH Regulation and the ECHA guidance on in vitro methods. In particular the guidance on aquatic vertebrates has a high likelihood to be the case for substances registered between 1 to 10 t/y. This poster will present an assessment of recent developments in the area of information generation for hazard identification, in particular focusing on alternative methods, such as (Q)SARs or in-vitro methods. The assessment is a consolidation of review papers, state-of-the-art papers and several recently finished/ongoing EU research projects on alternative approaches. Options will be presented for the new developments to meet information requirements (including as information below or beyond the minimum information requirements) for substances registered between 1 and 10 tones.

**MO 370**

Prediction of Michael-acceptor protein reactivity from quantum chemical reaction barriers  
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The so-called Michael acceptors, i.e. α,β-unsaturated carbonyls, are an important class of industrial chemicals with the potential to enter cells and react with endogenous macromolecules such as proteins and DNA. For this reason their toxicity toward the ciliate Tetrahymena pyriformis (logarithmic 50% growth impairment concentration after 48-h exposure, log EC50) exceeds the minimum or baseline toxicity as calculated from their hydrophobicity (logarithmic octanol-water partitioning coefficient, log Kow). It has been shown that their reactivity toward glutathione (GSH), quantified as logarithmic 2nd-order rate constant, log kcat,GSH can be employed to predict the toxicity component driven by their Michael-acceptor reactivity, log kcat,GSH a key determinant in predicting their toxicity.

The reaction of the electrophilic Michael-acceptor with the thiol group of GSH or protein side chains can proceed via two mechanisms. Firstly, a direct addition to the olefinic double bond (subject to our previous study), or, secondly, a conjugated 1,4-addition. In this work we present an extensive quantum chemical assessment of the 1,4-conjugated addition and the subsequent tautomerization step in simulated aqueous solution. For a set of 12 compounds, consisting of 4 aldehydes, 4 ketones, and 4 esters with different substituents at the C=C, double bond, reactant, transition-state, and product complexes with two explicit water molecules were calculated at the DFT and MP2 levels of theory. While DFT calculations yield ambiguous results as to which of the two steps is rate-determining, MP2 calculations support the textbook view that tautomerization is fast and not rate-determining.

The correlation of the intrinsic reaction barriers, AE, of the addition step with log kcat,GSH was explored, yielding a simple linear regression model with good statistics able to predict log kcat,GSH. In a second step, a regression model for log EC50 was developed, employing both AE and log Kow as descriptors. The derived models appear useful as in silico tools for screening the reactivity and toxicity of α,β-unsaturated carbonyls in the context of integrated testing strategies (ITS) for REACH, enabling an early waiving of animal tests for non-reactive new substances.

This work was financially supported by the European Union project OSIRIS (Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Testing Information), contract no: GOCCE-CT-2007-037017.
on the species level were reasonably well correlated pairwise (0.50<r<0.73) and showed no significant difference for all heavy metals. Thus, an aggregated heavy metal ranking was developed (IS_3), which assigns physiological sensitivity values to macroinvertebrates taxa. Considering the high variation, especially for higher taxonomic levels i.e. order, it is recommended to use S-values of the genus or species level for meaningful analysis. In terms of taxonomic ranking, crustaceans were found to be the most sensitive organisms, with species in the order of Cladocera being three orders of magnitude more sensitive than the most tolerant insects of the order of Trichoptera. By contrasts, molluscs covered a wide range of sensitivities, with Bivalvia being on an average one order of magnitude more sensitive than Gastropods. Finally, physiological sensitivity represents a promont trait that would help identify the effects of heavy metal pollution in lotic ecosystems, therefore improving practices in ecological risk assessment.

**MO 376**

**Identifying traits from arthropod community responses to insecticides**

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**INRA, Rennes, France**

An adapted version of SPEAR metrics for use in lentic experimental systems. LSPEAR (Lentic SPEArCies At Risk), has recently been proposed. In this study, LSPEAR was applied to two different pond mesocosm experiments to check if the invertebrate communities hosted by the systems were vulnerable and if this index could track their responses to pesticides. Data from two experiments previously run in 9-m³ outdoor tanks were used. During the first experiment, the ponds were allowed to mature for 1 year. Pond snails and Daphnia magna were introduced but snails did not successfully settled. Eight ponds were then introduced with deltamethrin and eight were kept as untreated controls. Four units within both the treated and untreated groups were covered with lids just after treatment to prevent external recolonization. The second experiment was performed after a six-month maturation period during which invertebrates collected from uncontaminated ponds were introduced. Among these, different crustacean taxa successfully settled the systems.

Exposure scenarios were built upon the modelling of transfer of pesticides applied on wheat and oilseed rape crops according to two crop protection programs. Different pyrethroid insecticides were applied, including deltamethrin. Abundance data were analysed using the multivariate Principal Response Curve (PRC) procedure. LSPEAR values in control and treated ponds were compared by ANOVA followed by post-hoc Tukey tests.

**MO 377**

**Application of a lentropic index to pond mesocosm experiments: why community assembly characteristics and season matter in ERA**

M. Rouqueca, A. Aubert, L. Lagadic, T. Caquet

**INRA, Rennes, France**

A several month-long experiment was implemented to assess the relevance of various parameters for the identification of direct and indirect effects of pesticides in aquatic ecosystems including the study of recovery periods. The same protocol was applied to outdoor dynamic and static experimental systems to assess if comparable results were obtained. In both experimental systems, it was anticipated that toxicology studies should be conducted to detect the presence of organisms from upstream and that lethal communities should be more resistant to toxic effects due to the selection of taxa exhibiting various traits associated with a higher tolerance to stressful environments (e.g., drying or low oxygen concentration).

Outflows (length: 40 m, depth: 50 cm) and ponds (volume: 7 m³, depth: 90 cm) were exposed to a dithiocarbamate fungicide, thiram. Two concentrations, process involves developing toxicological data for surrogate species. Surrogate species, often chosen because they are easy to culture, are supposed to be protective for many other species. For example, some regulatory agencies use toxicity data developed for one or two fish species to protect all fish and all amphibians. Because different, even closely related species can exhibit vastly different life history characteristics, surrogate species may not be representative of other species and may in fact result in under- or overestimation of ecotoxicological data for certain taxa. We will show how population models can be used to determine the life history characteristics necessary to protect groups of organisms. We will discuss a mathematical approach using insect biological controls and pesticides to determine whether one species can act as a predictive surrogate for others and what characteristics would make it protective. We will also present a generic model for the ERA of fish populations. The generic model was developed from five life history types and setup to be useful in a number of different management scenarios based on different environmental protection goals. A decision framework is set up for three levels of population models (continuous, time limited, and varying exposure) and three levels of environmental protection will be presented. The approaches presented here may guide future use of population models and surrogate species in ERA.

**MO 378**

**Comparative sensitivity of detritivores and detritus processing to Cu H.S. Kraak, E. M. Giller, M. Mulder, A.M. Bruins, W. Admiral**

**University of Amsterdam, Amsterdam, Nederland**

**RIVM LER, Biddhorn, Nederland**

**Agroecosystems and oriented organic carbon are central ecosystem processes that depend on the interactions between functionally diverse microbial and invertebrate communities. Ecosystem services are, however, under continuous pressure. Therefore the aim of the present study was to evaluate how toxicants may affect the functional links in the benthic food web and the services it is providing. To this purpose, the effects of the model toxicant Cu on functionally distinct macrofauna species (Asellus aquaticus and Tubifex spp.) and detritus processing were compared in microcosm experiments. Freeze dried, ground stinging nettle (Urtica dioica) served as detritus. After 5 days of exposure to Cu, detritus processing was determined by measuring LOI of the sediment and DOM content of the overlying water. Comparing the dose-response relationships for the effects of Cu on macrofauna survival and detritus processing revealed that the ecosystem process was much more sensitive to Cu than survival of the invertebrates. Spatial-temporal redox profiles of the upper 10 mm of the sediment showed that this was explained by a decreased activity of the invertebrates resulting in lower detritus breakdown rates at sublithal Cu concentrations. We conclude that detritus processing is a sensitive parameter for ecological water quality and ecosystem functioning.**

**MO 379**

**Comparative assessment of the structural and functional effects of a fungicide instatic and dynamic mesocosms**

Y.B. Bayona1, M. Rouqueca, C. Gorrizero1, A. Rouqueca1, K. Cailliaud1, A. Bassers2, L. Lagadic, T. Caquet2

**INRA Agrocosmo Ouest, Rennes cedex, France**

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**Washington State University, Puyallup, United States of America**

**University of Washington, Tacoma, Tacoma, United States of America**

The ability of ecosystems to recover from toxic pressures is dependent not only on exposure and innate susceptibility of individuals, but on the life history characteristics of the organism. Thus, species with certain life history characteristics (short generation times, early time to first production of offspring, and large numbers of offspring) should recover faster from stress than organisms with different characteristics if exposure and susceptibility are similar. The ecological risk assessment (ERA) process involves developing toxicological data for surrogate species. Surrogate species, often chosen because they are easy to culture, are supposed to be protective for many other species. For example, some regulatory agencies use toxicity data developed for one or two fish species to protect all fish and all amphibians. Because different, even closely related species can exhibit vastly different life history characteristics, surrogate species may not be representative of other species and may in fact result in under- or overestimation of ecotoxicological data for certain taxa. We will show how population models can be used to determine the life history characteristics necessary to protect groups of organisms. We will discuss a mathematical approach using insect biological controls and pesticides to determine whether one species can act as a predictive surrogate for others and what characteristics would make it protective. We will also present a generic model for the ERA of fish populations. The generic model was developed from five life history types and setup to be useful in a number of different management scenarios based on different environmental protection goals. A decision framework is set up for three levels of population models (continuous, time limited, and varying exposure) and three levels of environmental protection will be presented. The approaches presented here may guide future use of population models and surrogate species in ERA.

**MO 380**

**Life history characteristics of surrogate species used in ecological risk assessment: what is necessary for the protection of guilds? J.D. Stark, N. Hanson, J.E. Banks**

**Washington State University, Puyallup, United States of America**

**University of Gothenburg, Gothenburg, Sweden**

**University of Washington, Tacoma, Tacoma, United States of America**

The ability of ecosystems to recover from toxic pressures is dependent not only on exposure and innate susceptibility of individuals, but on the life history characteristics of the organism. Thus, species with certain life history characteristics (short generation times, early time to first production of offspring, and large numbers of offspring) should recover faster from stress than organisms with different characteristics if exposure and susceptibility are similar. The ecological risk assessment (ERA) process involves developing toxicological data for surrogate species. Surrogate species, often chosen because they are easy to culture, are supposed to be protective for many other species. For example, some regulatory agencies use toxicity data developed for one or two fish species to protect all fish and all amphibians. Because different, even closely related species can exhibit vastly different life history characteristics, surrogate species may not be representative of other species and may in fact result in under- or overestimation of ecotoxicological data for certain taxa. We will show how population models can be used to determine the life history characteristics necessary to protect groups of organisms. We will discuss a mathematical approach using insect biological controls and pesticides to determine whether one species can act as a predictive surrogate for others and what characteristics would make it protective. We will also present a generic model for the ERA of fish populations. The generic model was developed from five life history types and setup to be useful in a number of different management scenarios based on different environmental protection goals. A decision framework is set up for three levels of population models (continuous, time limited, and varying exposure) and three levels of environmental protection will be presented. The approaches presented here may guide future use of population models and surrogate species in ERA.

**MO 381**

**Behavioural response of juvenile rainbow trout during a short, low-dose exposure to a herbicide mixture**

**INRA, Rennes, France**

An adapted version of SPEAR metrics for use in lentic experimental systems. LSPEAR (Lentic SPEArCies At Risk), has recently been proposed. In this study, LSPEAR was applied to two different pond mesocosm experiments to check if the invertebrate communities hosted by the systems were vulnerable and if this index could track their responses to pesticides. Data from two experiments previously run in 9-m³ outdoor tanks were used. During the first experiment, the ponds were allowed to mature for 1 year. Pond snails and Daphnia magna were introduced but snails did not successfully settled. Eight ponds were then treated with deltamethrin and eight were kept as untreated controls. Four units within both the treated and untreated groups were covered with lids just after treatment to prevent external recolonization. The second experiment was performed after a six-month maturation period during which invertebrates collected from uncontaminated ponds were introduced. Among these, different crustacean taxa successfully settled the systems. Exposure scenarios were built upon the modelling of transfer of pesticides applied on wheat and oilseed rape crops according to two crop protection programs. Different pyrethroid insecticides were applied, including deltamethrin. Abundance data were analysed using the multivariate Principal Response Curve (PRC) procedure. LSPEAR values in control and treated ponds were compared by ANOVA followed by post-hoc Tukey tests.

During the first experiment, PRC only detected a transient effect of deltamethrin on invertebrates in open ponds, followed by a quick recovery. A strong effect of treatment duration of effects was categorized as in conditions of non-exposure. Analysis at the community level was performed using Principal Response Curves analyses (PRC). This analysis involved combination of PRC-axes. Additional multivariate techniques were used to investigate which autecological traits correlate to population level phenomena as initial impact and recovery potential. Furthermore, we investigated the relevance of traits in communities.

Finally, the relevance of the SPEA (Bayona & Esselink, 2011) for terrestrial arthropod ecosystems was discussed from the perspective of using ecological traits for prospecive or retrospective risk assessment in terrestrial ecosystems, similar to existing approaches developed for aquatic systems.
Fish are capable of sensing water-borne chemicals at concentrations below the lowest observed effect concentrations, which in turn are often several orders of magnitude lower than at the reported onset of mortality. In this study we exposed juvenile rainbow trout (Oncorhynchus mykiss) to an environmentally-relevant low-dose mixture of three co-occurring pesticides - atrazine, lithium and methachlor - during a 3-day test in controlled, flow-through conditions. Our hypothesis was that fish swimming activity, the food volume of the water column, and interactions between individuals would be modified due to exposure to the mixture.

Fish exposed to the mixture of pesticides were hypoactive and spent more time in the lower parts of the aquaria in comparison to non-exposed controls, reflecting inhibited swimming activity. Levels of aggression were comparable between the two treatments. In natural conditions, inadequate behavioural responses, such as excessive hypoactivity, could result in increased predation risk, as well as susceptibility to malnutrition through reduced foraging activity, with serious implications for survival.

Studies on the effect of pesticide mixtures are currently lacking, especially regarding the impact of environmentally realistic low doses on ecologically important and highly sensitive biological endpoints. Laboratory studies as the one presented here can contribute to the definition of the suitability of different behaviours for use in routine risk and hazard assessment. The behavioural endpoints chosen here were easily observed, and within a short timeframe, simple to quantify, and of biological significance and ecological relevance.

MO 382

Ecosystem functions vs. functional groups: do they respond in the same way to stress?

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Ecosystem responses to anthropogenic and environmental stressors on freshwater communities can propagate to ecosystem functions and may in turn impede ecosystem services. We investigated potential shifts in ecosystem functions that provide energy for freshwater ecosystems due to pesticides and salinity in 24 sites in streams of southeast Australia. First, effects on allochthonous organic matter (AOM) breakdown using three different substrates (leaves, cotton strips, wood sticks) in coarse and fine bags were investigated. Second, effects on stream metabolism that delivers information on stream ecosystem functions were measured. We found up to a fourfold reduction in AOM breakdown due to exposure to pesticides and salinity, where both stressors contributed approximately equally to the reduction. The effect was additive as, no interaction or correlation between the two stressors was found. Leaf breakdown responded strongly and exclusively to exposure to pesticides and salinity, whereas cotton stick breakdown was less sensitive and responded also to temperature changes such as nutrient concentrations.

MO 383

Effects of pesticide toxicity, salinity and other environmental variables on selected ecosystem functions in streams and the relevance for ecosystem services

R.M. Hedgespeth, J.A. Jonson, O. Berglund

Lund University, Lund, Sweden

Because pharmaceuticals and their metabolites have been detected in aquatic environments, there is potential for wildlife exposure and consequent effects on ecosystem structure and function. Not only is it important to examine sublethal effects such as changes in behavior of individuals, but it is also necessary to determine if these results in changes with broader ecological implications. This study focuses on the effects of pharmaceutical exposure on interactions between aquatic predators and prey, i.e. the European perch (Perca fluviatilis) and the small crustacean Daphnia magna. The effects of sertraline, a selective serotonin reuptake inhibitor (SSRI), on predator feeding rates were measured in different densities to examine changes in the predators’ functional response. This research will allow for application of ecological models to predict whether changes in predator-prey interactions caused by pharmaceuticals can alter population structures of aquatic organisms, and potentially community structure in aquatic environments. By taking such potential ecological impacts into account along with traditional methods, researchers and policymakers can improve the assessment of potential environmental risks of pharmaceuticals.

MO 385

The ecosystem services triad: linking stakeholder engagement, biophysical models, and ecological production functions to develop indices of ecosystem services for biodiversity

M.L. Hedgespeth, J.A. Jonson, O. Berglund

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There is tremendous momentum in the regulatory, academic, and business communities to develop approaches for measuring and managing ecosystem services. These efforts are evolving at various landscape levels ranging from site, to watershed, to regions. Most of these approaches rely to some extent on developing linkages between ecological processes and products and their use by humans. These linkages, often termed, ecological production functions, aim to describe and identify ecological inputs and processes that result in production of outputs that are valued by humans in such a way as to render ecologically meaningful measurement endpoints as well as to allow for accurate elicitation of values and preferences among stakeholders. One challenge for ecologists as well as social scientists is to develop analytical frameworks that accurately portray relevant ecological processes in ways that are easy to understand by non-scientists. Another challenge is to identify measurement endpoints that accurately represent ecological processes and functions, are easy and cost-effective to measure, and are good indicators of ecological changes over time. This poster will present results of a field demonstration of a decision support tool to quantify biodiversity-related ecosystem services at a U.S. Department of Defense site in the United States of America.

MO 386

Implementation of ecological risk assessment TRIAD for contaminated sites in the Basque Autonomous Community

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Tecnalia, Derio, Spain

The Ecological Risk Assessment (ERA) methodology currently applied in the Basque Autonomous Community (BAC) is based on Potentially Affected Fraction (PAF) % based on literature data and the concentration of contaminants on the site. However ecosystems are complex systems with multiple interactions and PAF may not be considered as a representative measure of ecological impact. With the aim of improving soil quality assessment methodologies in BAC, BERRILUR RTD project, pursues the implementation of TRIAD [1] as a proven ERA approach. Multiple expert groups from the Basque University and Research Centers are committed in BERRILUR gathering expertise from different disciplines. The first phase of BERRILUR has consisted in adapting the existing TRIAD approach to BAC regional conditions such as soil type, soil use and expert’s knowledge.

Results and information gathered were combined and three comparative tables were generated, one per LoF. In total, 18 chemical, 35 ecotoxicological and 62 ecological tests have been classified and prioritized. In each table the tests are classified according to the following scheme: (i) name of the test, (ii)organization that carries out the tests, (iii)taxonomic group and organism used in the test, (iv)type of contaminant (M. Metal, O. organic and T: all), (v)scoring of each test in each tier and finally, (vi)its corresponding ranking. So far results of the classification process show strong coherence, i.e. cheaper, quicker and highly standardized tests are ahead in tier 1, while the score decreases from tier 2 and 3. On the contrary, the tests in the latter positions of tier 1 are best positioned in tier 3. Regarding sensitivity of the tests towards nature of pollutant, slight differences could be appreciated only through chemical tests. The species used for testing consisted essentially in: microorganisms, plants, earthworms and slug. It should be noted that colomboLa, nematodes, arthropods and encrusted algae were not used in any test. So far, this means that available tests are not applicable to every existing soil function. It is therefore that we raise here the need to progress beyond the state of the art in by developing new testing tools and obtaining empirical data to assess specific replicability. In the second stage of the project the above methodology will be tested on a closed landfill in order to assess the ecological risk and define the possible soil uses and/or necessary remediation measures.

MO 387

Ecosystem services and environmental decision making: seeking order in complexity

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Fish tests have been classified and prioritized. In each table the tests are classified according to the following scheme: (i) name of the test, (ii)organization that carries out the tests, (iii)taxonomic group and organism used in the test, (iv)type of contaminant (M. Metal, O. organic and T: all), (v)scoring of each test in each tier and finally, (vi)its corresponding ranking. So far results of the classification process show strong coherence, i.e. cheaper, quicker and highly standardized tests are ahead in tier 1, while the score decreases from tier 2 and 3. On the contrary, the tests in the latter positions of tier 1 are best positioned in tier 3.
While there are still great uncertainties about the links between ecosystem biophysical drivers, biotic community structure and ecosystem functions and services, the concept of ecosystem services (ESS) is being applied in a rapidly expanding number of decision, management and policy frameworks. The majority of these frameworks can be referred to as “net environmental benefit analyses” (NEBA) or “net ecosystem service analyses” (NESA) as they seek to understand the net effect that various actions may have on ESS. As emergent properties of ecosystems, ESS are integrators of effects from multiple stressors and biophysical interactions at a range of spatial and temporal scales. ESS thus have the potential to provide a holistic approach using a “common currency” between environmental issues, helping as join up various programmes for a more integrated and coherently manageable approach. These frameworks are applied, defined, modelled and valued ranges widely between applications, potentially hindering their roles as cross-sectoral tools. For example, ESS can be transposed as assessment endpoints for “traditional” EoRA measurement endpoints; they can be assigned indices such as habitat suitability for comparative assessment; they can be quantified as measurable material or product flows; or they can be mapped, each with different scientific, economic, and societal implications. This paper will critically review how these issues are or could be addressed in a broad range of case studies in which ESS play a role, including examples for the assessment of environmental damage, sustainable remediation, International Finance Corporation Performance Standards, dredged material management, Integrated Coastal Zone, fisheries and landscape management, Plant Protection Products Registration, international environmental impact assessment, international due diligence, environmental insurance, and more strategic environmental decision making at catchment and landscape levels. Key assumptions, tools, similarities and differences will be identified, and opportunities for greater scientific relevance, clarity, transparency and read-across will be identified. Finally, the question of how we can ensure that current and evolving scientific understanding of ecosystem function and services in complex systems are relevant to and considered in wide-ranging environmental decision making and policy will be discussed.

MO 388

DASEES: a tripartite decision framework to achieve sustainable, Economy & society growth and management

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Many of Societies management and growth decisions are often made without a balanced consideration of pertinent factors from environmental, economic and societal perspectives. Particularly in areas where three key areas are present in many of the decisions facing societies as they strive to operate in a more sustainable manner. Typically decisions are made after considering one or two of these perspectives while ignoring the third, or the weighting for one or two of the perspectives vastly outweigh the second or third consideration. Far too frequently the process either ignores or does not adequately factor in consideration of the roles that ecosystem services play in a sustainable system. More specifically, decision-makers do not currently have access to useful or usable methods and approaches when they are presented with economic and social welfare choices that have significant ecosystem impacts. The goal of the USEPA Ecosystem Services Research Program (ESRP) Decision Support Framework (DSF) Team is to help provide that access by developing and delivering effective and user-friendly decision methods and approaches that decision-makers to explicitly, routinely, and substantively incorporate ecosystem services into their decision-making process. The goal of this framework is to provide decision-makers with an understanding of potential outcomes and effects of their planned decisions on economic, social, and ecological systems in order to promote more balanced and sustainable solutions. To this end the ESRP DSF team has developed a web-based multi-criteria decision analysis tool named NEMEA. Decision Support Framework (DSF) and Ecosystem Services Research Program (ESRP) integrates guidance and decision support tools into a five step iterative Bayesian decision process designed to: 1 - Understand the decision context; 2 - Define Objectives; 3 - Develop Options; 4 - Evaluate Options; 5 - Take Action. DASEES will be developed with stakeholder and decision-maker input, through case studies, to ensure the guidance, tools, and templates meet user needs and facilitate the incorporation of economic, societal and ecosystem services in the decision-making process.

MO 389

Acute exposure of common carp (Cyprinus carpio L.) fry to copper sulfate: a histological study

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One of the most common chemicals used in carp aquaculture is copper sulfate. It is regularly employed as a bath treatment against parasites as well as a fungicide and a herbicide. When used as a pesticide in plant treatment it can reach streams and rivers by surface runoff. It is known that copper sulfate is toxic, even in small quantities, and its use in fish farming has to be controlled. In this study, we investigated the histological changes in copper-exposed carp fry and their consequences for vital organs. In this experiment, 2 months old carp fry were exposed to increasing concentrations of copper sulfate (lower doses: 0.5 mg/L; 1 mg/L; higher doses: 2 mg/L; 4 mg/L) in aquaria for 1, 6, 12 and 24 hours. Fish fry were sampled, formalin-fixed, whole mounted in paraflin moulds and cut longitudinally for microscopic observations. The slides were stained histochemically with PAS (periodic acid-Schiff) or with propidium iodide and observed with light microscope and fluorescent microscope, respectively. Histological analysis showed various alterations in different organs of copper sulfate-exposed fry, especially the eye, the gills, the skin and the kidney. The structure of the eye was altered, it was partly shrunk and its function appeared to be impaired at higher doses. In the gills, complete fusion of secondary lamellec and necrosis of gill tissue were visible after exposure to higher doses of copper sulfate. Pathological changes in olfactory epithelium were seen after 12 and 24 hours, while lower doses induced less severe alterations: hyperemia, hypoplasia, hypertrophy, as well as lifting of the respiratory epithelium (after 12 and 24 hours). Most common change in the kidney was dilatation and degeneration of glomeruli, which was seen at higher doses and longer exposure periods (12 and 24 hours). Fragmentation of nuclei in skin cells was increased, indicating elevated level of apoptosis in the skin of copper sulfate-exposed fry. The liver and the intestine did not sustain histopathological changes and appear normal in all treatments. These results are consistent with earlier studies on the toxic effects of copper sulfate on fish, indicating dose-dependent alterations in the vital organs.

MO 390

Zebra mussel parasites: potentially useful bioindicators of freshwater quality?

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Parasites are major components of aquatic ecosystems in terms of diversity, ubiquity and key role in food web structure. This implies that they can be a reliable tool in assessing the effects of ecosystem disturbances, as an alternative or complement to macro-invertebrate approach. Their value as bioindicators of water quality has been documented with fish hosts, but few studies have focused on bivalves, particularly freshwater species. These host have the advantage of being less mobile and therefore provide information on the site-specificity and quality. In this context, we inventoried and analysed the composition and the structure of parasite communities of zebra mussel populations in twelve sites in France and the United States, characterized by different levels of contamination. Our specific goal was to determine if zebra mussel parasites could be used as bioindicators of freshwater quality. Each host population was characterized for parasite infection following standard histological methods. We used multivariate analyses and descriptive modelling to study parasite communities in relationship with environmental quality. Our results indicated that sites of different chemical contamination levels exhibited different parasite communities characterized by different trematode species and parasite associations. The contamination promoted infections by microsporidia (i.e. bacteria and algae), whereas it prevented those by macrosporidia (i.e. trematodes). For example, the occurrence of the unicellular bacteria, Rickettsiales-like organisms, was positively correlated with the nickel and chromium concentrations, and sampling indicated the absence of digenean trematode parasites at a highly contaminated site. Moreover, the use of multivariate analyses and modelling are promising tools to study zebra mussel parasite communities related to pollution

MO 391

Microcosms studies for evaluating potential of microbial community to degrade pollutants in soil and water ecosystems

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The ability of soil and water to recover from chemical contamination is primarily dependent on the presence of a microbial community with the ability to remove it. Functional specific microbial community characteristics are the main resource availability of ecosystem recovery and presence of pollution. In this way, the microbial community represents an important key to understanding the impacts of environmental and anthropogenic factors on ecosystems. Pollution can influence soil and water quality and productivity, but little is known on the effects on microbial communities, and consequent impacts on its functioning. Soil and water microcosms studies used for studying, under controlled conditions, the effects of selective pressures, such as the xenobiotic occurrence, on natural microbial communities. We report the overall results of several microcosm degradation studies, using natural soil and water samples, in which the degradation of many chemicals (including the parent compounds of pollutants (pharmaceuticals and their metabolites) in soil and water was evaluated, comparing the microbiologically active microcosms treated with the parent compound (e.g. pesticide or pharmaceutical) with others previously sterilized. The disappearance time of 50% of the parent compound (DT50) was evaluated for each chemical in the presence/absence of microbial community. The overall results presented here show the key role of communities in the degradation of the chemicals studied and encourage the use of microcosm approach for assessing more realistic environmental exposure scenarios and estimate the casual relationship between degradation of the role of microbial communities in chemical disappearance form the environment.
effects of veterinary medicines and on their risk to the environment has increased in the past years. The presentation gives an aggregated overview of the results of the environmental risk assessments performed at the German Federal Environment Agency since the coming into force of the guidelines. Needs for future basic research are identified. A summary of ongoing guidance development is given.

MO 396
Behavior of steroids and veterinary antibiotics in soil; study of transfer and degradation in soil columns
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Steroids are more and more used for animal treatments and are excrated unchanged. So, these compounds can enter the environment via land application of sewage sludge. This phenomenon could induce a competition for binding sites in human or animal medicine. Steroids, which are endocrine disruptors are also found in the environment due to animals but also humans wastes. The presence, distribution, fate and impact of veterinary substances and hormones regularly introduced into the soil via land application are far from known and very poorly characterised at the present time. In this context, the project aims at the development of methodologies based on soil columns experiments, in order to characterise the main products of biotic and abiotic transformations and to evaluate the migration and/or retention of selected contaminants and their breakdown products. For the selection of the compounds, the following criteria were selected: substances were selected for their biological activities, 2 metabolites were selected for their hormonal effects and 2 substances were selected for their antiviral effects. The degradation of the substances in soil was also studied. The kinetics of disappearance of the target contaminants were evaluated via modeling, by determining their concentrations in the different horizons and/or leachates were established. The influence of various parameters on this transfer was also examined such as the pluviometry and the composition of the soil (clay, loam, sand and organic matter contents).

The degradation of the substances in soil was also studied. The kinetics of disappearance of the target contaminants were evaluated via modelling, by determining their lifetimes and degradation constants. On the other hand, the presence, identification and quantification of the transformation products were considered by using complementary techniques of the coupling with two analysers: the time of flight (QqToF) and triple quadrupole (QqQ).

The radioactivity balance study of the storage experiment shows that the organic and acidic extractable radioactivity decreases over time. In contrast to these findings the non-extractable residues formed by SDZ and soil organic matter (NER) are increased considerably. To separate all the transformation pathways in one experiment, SDZ, Ac-SDZ and 4OH-SDZ were each labelled with different C isotopes and spiked to manure, on the field scale, the effect of temperature on the rate constants needs to be quantified. Therefore, fate dynamics were investigated at two different temperatures (10°C and 20°C). To separately observe all transformation pathways in one experiment, SDZ, Ac-SDZ and 4OH-SDZ were each labelled with different C isotopes and spiked to manure, which was then homogenously mixed with a silt loam soil. The batches were adjusted to 40% maximum water capacity and incubated in the dark for 150 days. Samples were repeatedly extracted with [13C]C12 and [12C]C12, with [3]acetonitrile-water, with [3]acetonitrile-water, with [13C]C12, with [13C]C12 and [12C]C12, with [3]acetonitrile-water, with [3]acetonitrile-water, with [3]acetonitrile-water. The extracts were analysed by HPLC-MSMS. The kinetic model was fitted to the concentration data. Model parameters were optimized by a stochastic optimization algorithm. All considered processes showed higher rate constants in the 20°C experiment. In principal, increasing temperature may exert different effects: (i) chemical and enzyme-catalyzed reactions are accelerated, (ii) microbial processes (including growth) are enhanced, and (iii) equilibrium sorption coefficients are shifted leading to larger dissolved compound fractions. In accordance, de-acetylation of [13C]Ac-SDZ was several times more rapid at 20°C than at 10°C. The same tendency was observed for the slow hydroxylation of [13C]SDZ, but the effect was not exactly quantifiable. NER formation could only be determined indirectly, but likewise seemed to be enhanced considerably with increasing temperature. Translocation into the residual fraction and back is assumed to be due to physical processes with less translocation than degradation. However, translocation also seemed to be positively correlated with temperature.

MO 397
Temperature dependence of transformation and translocation processes of manure-amended sulfadiazine in soil
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Sulfadiazine (SDZ) is a widely used veterinary antibiotic. In pigs, it is partly metabolized to N-acetyl sulfadiazine (Ac-SDZ) and 4-hydroxy sulfadiazine (4OH-SDZ). Fresh manure applied to agricultural soils contains a mixture of these three compounds. A conceptual kinetic model describing concentration dynamics of SDZ and its metabolites in soil considers hydrolysis of SDZ, de-acetylation of Ac-SDZ, irreversible formation of non-extractable residues (NER) and reversible sequestration into a residual fraction.

In field experiments with manure-amended SDZ, a dominant influence of temperature on the environmental fate dynamics was observed. For an application of the model on the field scale, the effect of temperature on the rate constants needs to be quantified. Therefore, fate dynamics were investigated at two different temperatures (10°C and 20°C). To separately observe all transformation pathways in one experiment, SDZ, Ac-SDZ and 4OH-SDZ were each labelled with different C isotopes and spiked to manure, which was then homogeneously mixed with a silt loam soil. The batches were adjusted to 40% maximum water capacity and incubated in the dark for 150 days. Samples were repeatedly extracted with [13C]C12 and [12C]C12, with [3]acetonitrile-water, with [3]acetonitrile-water, with [3]acetonitrile-water, with [3]acetonitrile-water, with [3]acetonitrile-water. The extracts were analysed by HPLC-MSMS. The kinetic model was fitted to the concentration data. Model parameters were optimized by a stochastic optimization algorithm. All considered processes showed higher rate constants in the 20°C experiment. In principal, increasing temperature may exert different effects: (i) chemical and enzyme-catalyzed reactions are accelerated, (ii) microbial processes (including growth) are enhanced, and (iii) equilibrium sorption coefficients are shifted leading to larger dissolved compound fractions. In accordance, de-acetylation of [13C]Ac-SDZ was several times more rapid at 20°C than at 10°C. The same tendency was observed for the slow hydroxylation of [13C]SDZ, but the effect was not exactly quantifiable. NER formation could only be determined indirectly, but likewise seemed to be enhanced considerably with increasing temperature.

MO 398
Fate of 14C-sulfadiazine in soil and characterization of its non-extractable residues
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RWTH Aachen University, Aachen, Germany
TU Dortmund, Institute of Environmental Research (INFU), Dortmund, Germany
Antibiotics are important therapeutics for the treatment of bacterial diseases in humans and animals. Most antibacterial compounds are metabolized only incompletely by the medicated animals and humans. Therefore biologically active compounds, besides partially active metabolites, are excreted in urine and feces. Manure originating from livestock is commonly utilized as agricultural feedstock. Therefore, antibiotics present in manure have been directly released into the environment for decades.

Sulfadiazine (N1-[2-pyrimidinyl]-sulfanilamid) belongs to the class of sulfonamides and is one of the most widely-used antibacterial veterinary products. It exhibits a wide spectrum against Gram+ and Gram- bacteria by inhibiting the p-pino benzonic acid in the folic acid metabolism. 

Non-extractable residues (NER) of xenobiotics in soil are discussed regarding their potential remobilization and bioavailability in the long term. In this study the fate of 14C-labeled sulfadiazine (SDZ) residues was studied in time-course experiments in soil. Silt loam was spiked with 14C-SDZ and amended with pig manure. Balances of 14C are based on mineralization, extractable and non-extractable residues. The main focus of this study was the characterization of the non-extractable residues associated with humic substances.

MO 400
A closer look on the non-extractable residues formed by SDZ and soil organic matter
S. Malcho, M. Lamshöft, S. Zähle, M. Spetliser
Institute of Environmental Research, Faculty of Chemistry, TU Dortmund, Dortmund, Germany
By means of radiolabelled veterinary drug sulfadiazine (SDZ) to soil and incubation for 200 days the formation of non-extractable and/or bound residues with soil organic matter is studied. The study focuses on the alteration of the extractable part of the soil organic matter which is referred to as humic acids. Extracted humic acids from the different soil samples are redissolved and separated utilizing size-exclusion-chromatography. Further analysis is carried out by radio-chromatography and high resolution mass spectrometry (HRMS).

The radioactivity balance study of the storage experiment shows that the organic and acidic extractable radioactivity decreases over time. In contrast to these findings the extractable radioactivity increases which means that the SDZ gets enriched in the humic acid fraction. The radio-chromatograms of the redissolved humic acids show two separated peaks. The first peak is broad and one estimated to be the SDZ which is not known contaminants. The second peak is sharp and is identified by mass spectrometry as free SDZ. This shows that a fraction of the non-extractable sulfadiazine is not bound to the humics. By quantification of the SDZ in these fractions and the evaluation of the results in context of the storage experiment the nature and dynamics of SDZ in soil can be characterized. This will lead to a further insight into the nature of binding of SDZ and in general xenobiotics to soil organic matter.

MO 401
Effects of amoxicillin, diclofenac and sulfadiazine on microorganisms and the nitrogen cycle in soil
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Veterinary drugs, e.g. antibiotics, enter the environment during fertilization with manure and may influence the soil organisms and nutrient cycles.

The present work summarizes information on the effects of different antibiotics added with pig manure on soil microorganisms and the nitrogen cycle pathways (potential
Determination of the veterinary antibiotic erythromycin's potential to bioconcentrate in the aquatic invertebrates, Lumbriculus variegatus and Daphnia magna, and evaluation of their bioavailability in dairy manure

T.K. Wings1, F. Platzbecker2, W. Dott3

RWTH Aachen University, Aachen, Germany

The project is subdivided into three subsequent phases:

1. Method development using cattle manure of different origin and the VMP salicylic acid. Method development includes an intra-laboratory validation. Fate of salicylic acid is described by dissipation of the parent, formation or characterization transformation products, NER, CO2 and CH4 mass balances. The fate is compared to 0.13 for the 5-ppm treatment.

Still crucial points are: the influence of a static versus a flow-through incubation system on the ratio of formed CO2 and CH4, suitability of methods to determine the microbial activity (using 14C-glucose as reference substance in comparison to the fumigation method used for aerotrophic transformation studies), and the influence of storage temperature and storage duration on the test manure.

MO 405
Co-extraction and -quantification of 16 veterinary antibiotics and the evaluation of their bioavailability in dairy manure

T.K. Wings1, F. Platzbecker2, W. Dott3

RWTH Aachen University, Aachen, Germany

The experiments were performed to determine the partitioning of the compounds through day 14.

These organisms yield higher BCF values at disease-expansion to an entire livestock. But the continuous feeding of antibiotics demonstrably leads to the dispersal of antibiotic substances in manure, soil and ruminant waterbodies thus creating the selective pressure of constant subtherapeutic levels which are known to promote the spread of resistant and potentially pathogenic microorganisms. Accordingly the initial goal of this work was to develop a method for the co-extraction and subsequent quantification of 16 representatives of the most notable veterinary medicinal classes (350t tetracyclines, β-lactams 199.2t, 97.5t sulfonamides, 52.6t macrolides and 3.7t fluoroquinolones in 2005) in manure. The Extraction of antibiotics from manure was done using a McMellon buffer. Purification and concentrating of the analytes were accomplished through co-polymer-based solid phase extraction (SPE). The chromatographic determination, using UPLC-MS/MS, ran over a BEH C18 column with an acetonitrile gradient. The antioxidant properties of the compounds were identified from retention time and fragmentation pattern of each analyte using MS/MS and quadrupole mass spectrometry and detection by MS/MS in MRM mode. The detection limits for the MS/MS in dairy manure were determined at 0.4 μg/kg. The recoveries between 28% and 158% were determined in dependence of the analyte and its concentration. For the investigation of the antimicrobial activity and bioavailability of the individual compounds an agar diffusion test was used. The results showed a significant inhibition of antibiotic potential under the influence of manure matrix in contrast to application of the pure substance. Further work will provide an extended method for the use on livestock manure, due to a dramatically higher antibiotic consumption and correspondingly higher residues that can be expected.

MO 406
Determination of the veterinary antibiotic erythromycin's potential to bioconcentrate in the aquatic invertebrates, L. variegatus and Daphnia magna, and evaluation of their bioavailability in dairy manure

T.K. Wings1, F. Platzbecker2, W. Dott3

RWTH Aachen University, Aachen, Germany

The selected experimental setup is a suitable basis for studying transformation of VMP in dairy manure. The test design gives reliable and reproducible results. Statistical analyses yield minor variations in DT10 and DT50-values of the test substance. Also, quantification and characterization of transformation products as well as determination of formation of NER, CO2 and CH4 is performed.

Still crucial points are: the influence of a static versus a flow-through incubation system on the ratio of formed CO2 and CH4, suitability of methods to determine the microbial activity (using 14C-glucose as reference substance in comparison to the fumigation method used for aerotrophic transformation studies), and the influence of storage temperature and storage duration on the test manure.

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Determination of the veterinary antibiotic erythromycin's potential to bioconcentrate in the aquatic invertebrates, Lumbriculus variegatus and Daphnia magna, and evaluation of their bioavailability in dairy manure

T.K. Wings1, F. Platzbecker2, W. Dott3

RWTH Aachen University, Aachen, Germany

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MO 407 Determination of the antiparasitic pharmaceutical tetracyclines and two metabolites in environmental water, soil and animal manure
J. Olsen, M. Hansen, E. Bjorkland, K.A. Krogh
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Tetracyclines (TCs) and sulphonamides (SFs) are broad-spectrum antibacterial agents, and antibiotic resistance in Enterobacteriaceae is among the most important problems in veterinary medicine. The presence of TCs in the environment is one of several possible factors that may cause antibiotic resistance in enterobacteria. A novel method was developed to determine TCs and SFs in swine wastewater and river water using deuterium-labeled TC and SFs as internal standards. The method is based on solid-phase extraction and ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS).

The sensitivity of the method was comparable to or better than that of the previously reported methods. The concentrations of TCs and SFs were determined in river water, swine wastewater and animal manure, and were below the limits of quantitation. The results indicated that the exposure of aquatic environments and livestock to TCs and SFs is low, and that the current use of these compounds is not a major contributor to antibiotic resistance in enterobacteria.

MO 408 Determination of tetracyclines and sulphonamides in swine wastewater
J.M. Lin
Institute of Environmental Health, Taipei, Taiwan

The animal husbandry industry provides a stable source for livestock products. There is a huge demand for veterinary antibiotics in concentrated animal feeding operations (CAFOs) on treating and preventing diseases as well as on promoting growth. This practice may favor a selection of antibiotic-resistant genes. To evaluate the potential impact of emitting antibiotics on the environment, a good analytical method is desired for measuring veterinary antibiotics in environmental matrices.

Tetracyclines (TCs) and sulphonamides (SFs) are widely used as drugs to treat infections in animals. In the production of swine and poultry, TCs and SFs are used as feed additives. In this study, a method was developed to determine TCs and SFs in swine wastewater and river water using deuterium-labeled TC and SFs as internal standards. The samples of influent and effluent were prepared with solid-phase extraction (SPE), and were analyzed using UHPLC-MS/MS at positive electrospray ionization. The results showed that TCs and SFs were detectable in the influent and effluent, and that the concentrations of TCs and SFs in the influent were 40-75 mg/L and 150-250 mg/L, respectively, which were similar to those measured in other studies. This study demonstrated that the concentrations of TCs and SFs in swine wastewater and river water can be monitored using the developed method, and that the method is suitable for environmental monitoring of veterinary antibiotics.
The field samples needed for sediment assay were taken using the Kajak sediment core sampler. In the semifield assay, special cylinders were used, which contained the 5 cm sediment layer and 10 cm of water column spiked with tested antibiotic. Two growth parameters of L. minor were measured: frond number and area. Concurrently, the concentration of tetracyclines in water column was monitored with HPLC-DAD.

The aim of the present study was to investigate the influence of temperature variation on the toxicity of the veterinary antibiotic florfenicol to the freshwater grazer Daphnia magna. The assays were carried out for 24 h at three different temperatures (15, 20 and 25°C), in the presence and absence of light with juveniles and adult females using feeding inhibition as effect criteria. The results indicate that temperature effects of florfenicol on D. magna feeding behaviour highlighting the need of more research on the combined effects of temperature and pharmaceuticals on freshwater grazers.

This work was supported by EU-FEDER and national MCTES funds through a PhD grant from the Portuguese Foundation for the Science and Technology (FCT) to Alexandra Martins (SFRH/BD/65436/2009).

MO 414

Histological changes in fish Astyanax altiparanae) exposed to 17-Ahpha-Methyltestosterone

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3WUR, Alterra, Wageningen, Netherlands

The use of antibiotics to prevent and treat bacterial infections in aquaculture has raised considerable concerns because of their potential environmental release and the potential effects on the structure and functioning of surrounding aquatic ecosystems. The majority of the available studies have this issue focused on marine aquaculture produced in temperate and cold regions, whereas the great bulk of the global aquaculture production takes place in tropical and sub-tropical regions of Asia. In the present study, the use of antibiotics in tilapia cage production in the Tha Chin river (Suphanburi Province, Thailand) was investigated. Semi-structured questionnaires were performed in 15 farms between the 6th and 22nd of April of 2011. All the interviewed farmers reported to have used antibiotics during the last year with enrofloxacin (73.3% of the farms), followed by florfenicol (53.3%), tylosin (16.7%), oxytetracycline (60.0%) and amoxicillin (40.0%). The results of analysis revealed that measured concentrations of individual antibiotics were significantly different depending on sampling location. Chlorotetracycline (CTC) was not detected in any of the samples. High concentrations were mainly found in urbanized regions of the Istanbul Strait. The field samples needed for semifield assay were taken using the Kajak sediment core sampler. In the semifield assay special cylinders were used, which contained the 5 cm sediment layer and 10 cm of water column spiked with tested antibiotic. Two growth parameters of L. minor were measured: frond number and area. Concurrently, the concentration of tetracyclines in water column was monitored with HPLC-DAD.

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MO 415

Use of antibiotics in tilapia cage aquaculture: a case study in the Tha Chin river (Thailand)

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The use of antibiotics to prevent and treat bacterial infections in aquaculture has raised considerable concerns because of their potential environmental release and the potential effects on the structure and functioning of surrounding aquatic ecosystems. The majority of the available studies have this issue focused on marine aquaculture produced in temperate and cold regions, whereas the great bulk of the global aquaculture production takes place in tropical and sub-tropical regions of Asia. In the present study, the use of antibiotics in tilapia cage production in the Tha Chin river (Suphanburi Province, Thailand) was investigated. Semi-structured questionnaires were performed in 15 farms between the 6th and 22nd of April of 2011. All the interviewed farmers reported to have used antibiotics during the last year with enrofloxacin (73.3% of the farms), followed by florfenicol (53.3%), tylosin (16.7%), oxytetracycline (60.0%) and amoxicillin (40.0%). The results of analysis revealed that measured concentrations of individual antibiotics were significantly different depending on sampling location. Chlorotetracycline (CTC) was not detected in any of the samples. High concentrations were mainly found in urbanized regions of the Istanbul Strait. The field samples needed for semifield assay were taken using the Kajak sediment core sampler. In the semifield assay special cylinders were used, which contained the 5 cm sediment layer and 10 cm of water column spiked with tested antibiotic. Two growth parameters of L. minor were measured: frond number and area. Concurrently, the concentration of tetracyclines in water column was monitored with HPLC-DAD.

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MO 416

Tetracycline and Fluoroquinolone concentrations in the Istanbul strait sediments

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2Research Center for Eco-Environmental Sciences, Beijing, China

Chlorotetracycline (C1T) and oxytetracycline (OXY) have been detected in the marine environment at high levels, whereas the great bulk of the global aquaculture production takes place in tropical and sub-tropical regions of Asia. In the present study, the use of antibiotics in tilapia cage production in the Tha Chin river (Suphanburi Province, Thailand) was investigated. Semi-structured questionnaires were performed in 15 farms between the 6th and 22nd of April of 2011. All the interviewed farmers reported to have used antibiotics during the last year with enrofloxacin (73.3% of the farms), followed by florfenicol (53.3%), tylosin (16.7%), oxytetracycline (60.0%) and amoxicillin (40.0%). The results of analysis revealed that measured concentrations of individual antibiotics were significantly different depending on sampling location. Chlorotetracycline (CTC) was not detected in any of the samples. High concentrations were mainly found in urbanized regions of the Istanbul Strait. The field samples needed for semifield assay were taken using the Kajak sediment core sampler. In the semifield assay special cylinders were used, which contained the 5 cm sediment layer and 10 cm of water column spiked with tested antibiotic. Two growth parameters of L. minor were measured: frond number and area. Concurrently, the concentration of tetracyclines in water column was monitored with HPLC-DAD.

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MO 417

On-line SPE-LC-MS for ultra trace analysis of antibiotics in river and ground water

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Endocrine disrupting compounds (EDCs) are a broad range of natural or synthetic chemical substances, mostly of anthropogenic origin. Some typical representatives of EDCs are industrial chemicals, cosmetics, pesticides, hormones, medical products and many common pharmaceuticals. Among the latter group, antibiotics are of a special interest as their presence in the - mostly aquatic - environment is a potential cause for antibiotic resistance of many bacteria. Obviously, the concentrations of these compounds in environmental samples are very low, i.e. in the µg/L scale (ppb to ppt range) which is a real challenge for analysis. An analytical method for the analysis of selected antibiotics in aqueous matrices like river water is presented. It comprises a sample clean-up and pre-concentration procedure combined with solid phase extraction (SPE). The intended system is fully automated and performed on-line with the HPLC-MS step in an integrated set-up with column switching abilities and under single point software control. The feasibility of the system will be demonstrated by analyzing a selection of representative antibiotics, mostly from the tetracycline and macrocyclic type.

RA040P - Established and emerging footprints – striving towards a valid and comprehensive support for decision-making processes

MO 421

Does ‘green power’ purchase affect Carbon footprint?

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Which electricity models to be used for Carbon footprint is often debated; e.g., how is “national grid” understood and defined? However, when introducing Guarantee of Origin (GO) and Electricity Disclosure the according to the European Renewable Energy Directive (2009/28/EC) and Electricity Market Directive (2009/72/EC), respectively, as well as US Renewable energy Certificates, the issue gets more complicated.

A GO is defined as a means of proving the origin of electricity, while the objective of the Electricity Disclosure is to provide consumers with relevant information about power generation and to allow for informed consumer choice, not only based on electricity prices. The suppliers of electricity are required to disclose their electricity portfolio with regards to energy source and environmental impacts, specifying the emissions of CO2 and the amount of radioactive waste. Consumers can make an active choice regarding the environmental performance of the purchased electricity by voluntarily purchase a GO. In US Renewable energy certificates (RECs), represent a similar voluntary product. RECs represent the environmental attributes of the power produced from renewable energy projects and are sold separate from commodity electricity.

Several standards and guidelines (e.g. ISO14067 Carbon footprint of products, GHG Protocol) are working on general rules for how such products should be interpreted in Carbon footprint calculations. The aim of this presentation is to show how different ways of dealing with this issue affect the Carbon footprint calculations. There seems to be a need for common guidelines for the interpretation of GOs/RECs in Carbon footprint calculations are needed to be able to make fair comparisons between products and services.

MO 422
Climate impacts of transitioning from coal to natural gas fired electricity generation over time
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Transitioning from coal-fired electricity generation towards a greater share of natural gas (NG) is increasingly perceived to substantially mitigate climate change due to lower life cycle Greenhouse gas (GHG) emissions. Life cycle assessment (LCA) is an accounting tool that compares technologies based on the GHG emissions over their life cycle. The life cycle GHG balance of a specific technology does not represent actual climate impacts because (1) it is based on a per energy basis, and (2) using GHG emissions as a metric the physical processes leading from GHG emissions to climate change and climate impacts. The physical processes include the generation of radiative forcing (RF), the time lag between RF generation ( delay time) and the time lag of climate changes (AT), which are included in the LCA but not represented in the life cycle GHG emissions.

We apply life cycle emissions distributions of coal and NG fired electricity generation to a stochastic climate model to quantify the absolute climate impacts of different transitioning scenarios. We extend current models by explicitly modeling two major sources of uncertainty. First, we bound bottom-up life cycle CH4 emissions from leakage during NG production using top-down atmospheric measurements. Bounding is given important the current wide distributions of CH4 leakage in the literature and the resulting controversy regarding the correct leakage rate. Second, transient climate sensitivity, which accounts for the magnitude and temporal distribution of the climate change response to GHG emissions, is represented stochastically in the climate impact module. Quantifying uncertainty in the climate response is vital for drafting policy recommendations that go beyond GHG emissions by addressing climate change directly.

Results show that substituting coal with NG provides little if any climate benefits despite significantly correcting the CH4 leakage to lower levels. Assuming that U.S. power generation from NG increases by 25% beyond current projections until 2035, thereby reducing coal power by the same absolute amount, RF will be reduced by only about 1-3% over 25-100 years (0.0003-0.0007 W/m²). Moreover, if reduced coal power generation in the U.S. is used to meet coal demand elsewhere, the coal-NG substitution may actually increase total RF. Current work aims to quantify the influence of climate model uncertainty on these results.

MO 423
Reducing energy and GHG footprints of microalgae biodiesel by the use of co-products from the sugarcane ethanol industry
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1Universidade Estadual de Santa Cruz, Ilhéus, Brazil
2Universidade Federal de Viçosa, Salvador, Brazil

Greenhouse gases (GHG) and energy assessments are useful techniques to assess environmental impacts resulting from the distinct stages of an industrial practice. Such stages may include upstream and downstream processes in addition to the main activities carried out during the manufacturing of a specific product. In this study, Life Cycle Energy and GHG Assessment (9, 10) were carried out in order to compare the environmental impact of biodiesel production from microalgae cultivated in open ponds. Microalgae from microalgae is being considered today as a superior alternative to land-crops for the production of biofuels. Comparatively, cultivation of microalgae offers a better potential for increasing biodiesel productivity and improving the capacity for CO2 sequestration (4, 5). This study considered two distinct scenarios in a pilot-scale production of microalgae cultivation for biodiesel production.

Brazil is an important worldwide player in the biodiesel industry with an installed productivity capacity of 5,5 million liters per year (3). The use of biodiesel in Brazil has been favored due to the availability of agricultural land. However, the expansion of oil-seed crops may lead to land use changes which in turn favor the release of CO2 and nitrogen gases stored in soil (1). The production of biodiesel from microalgae does not show such disadvantage.

The default scenario: (i) biodiesel production based on microalgae biomass outputs, in a system using commercial energy, CO2 and chemical fertilizers as nutrient sources. In this scenario, the algal cake is also submitted to pyrolysis for the production of oil, gas and biochar; was compared with: (ii) the same conditions as described in the former item (i), but now using CO2 and biodigested vinasse from the sugar cane industry to supply part of the fertilizer demand of the algae, the other part being supplied by the digestate of the anaerobic digestion of algal cake.

Life Cycle Energy and Carbon Footprint Assessments allow the identification of possible synergistic aspects of algal biodiesel production and the sugarcane ethanol industry, concerning a future integration of the two production system. The preliminary results indicate significant positive effects on the energy and carbon footprints of biodiesel of using CO2 and biodigested vinasse from the sugar cane industry to supply part of the fertilizer demand of the algae, the other part being supplied by the digestate of the anaerobic digestion of algal cake.

MO 424
Including urgency in global warming assessment in LCA
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Climate change mitigation is more relevant today than ever, as clearly illustrated by the conclusions in the World Energy Outlook 2011 just published by the IEA, giving us only until 2017 to take action in order to stay below a global warming of 2 degrees Celsius.

However, climate change mitigation issues are not new and have already been discussed in the 90’s. Currently, scenarios on climate change today are still widely discussed. At midpoint level the use of the global warming potential (GWP) is completely dominating, but a major short coming of the GWP is the lack of consideration of short term issues, such as important climatic target times - the points in time where climatic tipping points are expected to be crossed. Several alternative metrics to the GWP have been suggested in recent literature. Some include considerations of short term impacts occurring beyond such target times. Addressing both of these issues are essential in the pursuit of mitigating climate change, however they should be addressed separately, as they represent two different aspects of the climate change challenge.

This paper introduces an additional impact category in life cycle assessment (LCA), called the climatic tipping point impact category, addressing the urgency related to prevent the crossing of such target times. The new category is called Climate Change Impact Category (CCIC) and will integrate two of the existing impact categories. Once the climate change impact is reached, the effect of such climate change impact is greater and the due to the short term issues. Furthermore, is it necessary to introduce a new impact category follow-up tipping points.

This category will take into account the situation of climate change beyond the 2030 target, which can be better described by the category of Climate Change Impact Category (CCIC).

Acknowledgement: The research was funded by the Key Projects in the National Science & Technology Pillar Program during the 12th Five-year Plan Period “Research on carbon footprint of typical products of electronic information, paper making and printing” and “Research on the key technique for certification of carbon emission and carbon reduction”.

MO 425
Study on the community CO2 emission based on LCA in Beijing
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This research assessed the size and major components of a community carbon footprint based on the life cycle assessment approach combined with IPCC methods. Data related to CO2 carbon footprint were collected, which included household energy consumption, public and car transportation, waste production, etc. The result showed that energy consumption in electricity consumption was more than 60% of CO2 emission, with transportation and waste production resulting in a further 24% and 10% of total emissions, respectively. The carbon absorbing effect of public green space was analyzed. As the result showed, the public green place in the community reduced about 25,262kg CO2 emission every year. According to the status of Beijing, the proposals for reducing the CO2 emission and the low-carbon community continuing to be build forward.

Acknowledgement: The research was funded by the Key Projects in the National Science & Technology Pillar Program during the 12th Five-year Plan Period “Research on carbon footprint of typical products of electronic information, paper making and printing” and “Research on the key technique for certification of carbon emission and carbon reduction.”

MO 426
How valid and comprehensive is our CED practice today? I.S. Modahl, K.A. Lyng, H.L. Raadal, C. Aashkel
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Several of the carbon footprint indicators developed, while others, such as carbon footprint and cumulative energy demand (CED), are more or less regarded as fully developed and integrated into conventional LCA studies. This paper will, however, focus on methodological gaps and challenges such as double counting and missing characterisation factors used for this ‘fully developed’ environmental indicator indicator CED.

These challenges are connected to the following aspects:

- Use of specific data (for example for energy carriers with a heat value different from the pre-defined energy carriers in the database and characterisation methods used).
- Feedstock issues:
  - How do you define the raw material used for building a wooden shed?
  - Should there be any change somewhere in the life cycle, if you extend the analysis from cradle-to-gate to cradle-to-grave, when using the wooden material as an energy source after dismantling?

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The methods for calculating cumulative environmental demand depend on the correct input, in the correct form, for calculating comparable indicators. In contrast to calculating carbon footprint, the ‘correct’ input for calculating CED is heavily dependent on the LCA practitioner being aware of how the LCI indicators are calculated, and the practitioner’s knowledge of how to manipulate the pre-defined CED method. In addition, the different CED methods available in LCA software tools are not always implemented with awareness of the challenges connected to feedback issues. It is also interesting that older CED methods seem to be more accurate than newer ones, due to the inclusion of more substances.

The paper will illustrate these challenges by describing quantitatively the inconsistencies introduced by using specific energy carriers and raw materials which can both be used as feedstock and energy sources. The aim of the paper is to raise awareness of the CED challenges and to improve current practice in order to increase the accuracy of the CED results.

MO 427
Development of water inventory database considering water type and form of water use
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Waterfootprint (WF) is paid attention by water resource depletion and eccentric water. WF can be expressed as total amount of water used at entire life cycle of products. WF is expected to be effective the following hour. First WF can understand the magnitude of the impact of water use. Second risk management strategies for water use. Third provide an optimized water management at the organizational level. Finally Communication and marketing strategies. WF is discussed for standardization in ISO. According to ISOWD, they require quantity of water used, type of water used, form of water use, geographical location of water withdrawal and return in water inventory. Though these database are not exhaustive, this study developed about 3700 sector water inventory database considering water resources. WF are expressed in output table in Japan. As characteristics of database, in the input aspects water is classified into river, ground and reused water, and consumption aspects it has three types: rain, river and ground water. Based on the database, annual water use by one average family in Japan and import and export water have been calculated.

MO 428
Conceptual framework for grey water footprint of copper mining production
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Given the concerns about global water resources and the particular situation of aridity and water scarcity in the main mining areas of Chile, a priority for mining industry is to advance towards an effective sustainable management resource of water. The grey water footprint is defined as the volume of freshwater that is required in order to assimilate the load of pollutants discarded a water body taking into consideration the natural concentrations and the existing ambient water quality standards. This work aims to develop a conceptual framework to adequately measure a relevant sustainability impact indicator for the copper mining production through the grey water footprint method, which is one of the most widely used methods for assessing the consumptive use of water. Therefore, the study focuses on developing a framework for accounting the grey water footprint and establishing the most critical pollutants in the effluents of the copper cathodes production, of both the sulfide and oxide production lines, according to the water footprint method. The methodology of life cycle assessment was applied to establish the system boundaries, the calculation basis and the allocation criteria.

MO 429
Closing methodological gaps of water footprint: scope and aggregation issues
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While for many impact categories such as GHG emissions and fossil resource consumption indicators with long experience exist, for water resources impact assessment the knowledge is still limited and bears the additional problem of high spatial variation. The integration of the different aspects of water impacts into a single water footprint metric is a required for proper and robust communication, also addressing the different levels of uncertainties. Also there is a relevant discussion about what impacts should be included in a water footprint in order to avoid double counting and to have a meaningful footprint. In this project, all impacts related to emissions to or consumption of freshwater are included. While for substance emissions to water many sophisticated model exist, there is still a lack of proper assessment of heat releases and anti-fouling detergents that are crucial for thermal power production. These impacts have been addressed and integrated.

In a second step, we developed a scheme to combine the various existing LCIA methods related to water resources on midpoint level of the LCIA factors as well as on endpoint level for ecosystem quality impacts. For illustrating the issues related to aggregation and communication of such a water footprint, we developed specific indicators for cooling water technologies and state of the art coal and gas power plants, including regional differentiation of the supply chain and corresponding geographies for the impact assessment, creating maps of aggregated water footprints.

More detailed analysis of the results revealed that the aggregation step and hence the final metric is very sensitive to underlying assumptions, aggregation procedures and the coverage of included methods. Although in the ISO 14040 series full aggregation of impacts is not allowed for public reporting, the demand for water footprint labels requires a single number and therefore our results are accounting for and reporting the different uncertainties involved.

MO 430
Evaluation of a range of impact methods for assessing water quantity and water quality for a liquid laundry product
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A large number of impact assessment methods have been proposed for measuring the water quantity and use aspects of products. Twenty methods including USEPA, grey water from the Water Footprint network and midpoint indicators based on Water Stress Index (WSI) were applied to a case study of a European liquid laundry product. The results of the study were to: 1) understand the strengths and limitations of each method; 2) evaluate their informational requirements and identity gaps in the data available for the life cycle of a detergent product; 3) contrast potential impacts related to water quantity and water quality at the midpoint and endpoint level and 4) provide some initial insights in understanding the water flows of the products system through a mass balance at the inventory level. The case study was based on a concentrated liquid detergent used in France. The functional unit was one wash at 40oC. Life cycle inventory data was obtained from ecoinvent and the Quantis Water Database. The life cycle stages taken into consideration were chemical supply, manufacturing, consumer use, and end of life (wastewater treatment and packaging disposal).

The quality of the results is directly linked to the quality of the data used in this evaluation. For example there were data gaps in the primary data collected e.g. country of origin of the raw materials and therefore assumptions were made. Typically the consumer use stage dominates the impact scores for 15 out of 20 impact assessment methods applied.

MO 431
Water assessment of a hand dishwashing product using an LCA approach: opportunities and limitations
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This study demonstrates the state of the art both from a database and a method perspective. The study suggests that the consumer use stage is the most important one (99% using the Ridoutt and Pfister method), driven by both direct water use (dishwashing process) and water footprint (water use associated with raw materials supply chain). The study also demonstrated that the state of the art both from a database and a method perspective.

The study focused on environmental issues related to water use, looking in depth at inventory methods, midpoint and endpoint methods (14 in total) and covered the entire life cycle of the product. The use of a single product with the same global supply chain, sourced from one location was evaluated in two countries with a different water scarcity conditions. The study suggests that the consumer use stage is the most important one (99% using the Ridoutt and Pfister method), driven by both direct water use (dishwashing process) as well as indirect water use (related to the electricity to heat the water). Therefore, the largest improvement opportunity is to build consumer awareness on habits and practices in water scarce regions. The study also showed a very different profile if spatially explicit methods are applied on one product used with the same use conditions. From a methodological approach, a lot more work is necessary to develop good inventory databases (in terms if flows covered and accurate data), which is key for all methods to deliver the necessary for the application of water assessment methods in a business context. A number of the applied methods are not regionalized, which was demonstrated to be a critical aspect in comparing the overall water profile of the product. Final improvements to some methods are suggested.

MO 432
Science behind biodegradable claims on down the drain consumer products
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This poster provides an overview of various approaches that could be used to evaluate the biodegradability of consumer products. The biodegradability of formulations is increasingly perceived as a key attribute for modern “sustainable” products. Despite biodegradability becoming a common environmental claim on the packages of consumer goods products, there is no standard technical approach to assess the biodegradability potential of formulations (i.e. complex mixtures, preparations, finished product, whole-product formulations). This creates the potential for misleading or even incorrect claims. For example, biodegradation is only relevant for organic constituents and...
for inorganic ingredients (e.g. water). Biodegradability assessments are typically required by law for the hazard and risk environmental assessments of individual organic substances. The existing standard procedures (e.g OECD 301 Testing Guidelines) for ready biodegradability are only appropriate for individual organic chemicals or mixtures of similar chemicals (i.e. closely related homologues). The OECD guidance specifically states that these tests are inappropriate for mixtures of dissimilar substances as would exist in a product formulation. Hence, ready tests of whole products are insufficient for establishing the full biodegradability of a product formula. Furthermore, even when one has appropriate biodegradation data on the individual ingredients in a formula, different rules and calculation methods can yield quite different results. For example, the percent biodegradability for a single formulation can vary from <70% to > 90%, depending on whether the percentage is based upon the total number or mass of ingredients as well as if all constituents or only those constituent for which biodegradability is a relevant attribute are included in the calculation. We evaluated the pros and cons of each approach and conclude that the most scientifically rigorous approach is to calculate percent biodegradability of a formula based upon the mass of only those ingredients for which biodegradation is relevant. Going forward, there is a need for a common scientifically based approach and internationally accepted calculation method to assess the biodegradability of whole product formulations to safeguard the credibility of environmental claims among the general public and consumers organizations.

MO 433 Development of impact assessment for chemical substances covering PRTR substances
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The problems of existing assessment methods for chemical substances in LCIA are the lack of covering chemical substances and that of reliability in model. The approach has been developed to develop an indicator for chemical substance evaluation. The above problems will be observed in order to overcome them. Concretely, the approach of this research are to develop Characterization factors on ecosystem for chemical substances prescribed by PRTR/Pollutant Release and Transfer Register in Japan and to develop those on ecosystem and human health using USEtox that is consensus model for LCIA developed by UNEP / SETAC Life Cycle Initiative. This research was developed based on the knowledge gained from comparative verification by developers of existing evaluation method. Furthermore, this research will be aimed at developing those corresponding to the evaluation in Asia have currently been spreading SCA method. The brief process of this research can be described as follows.

1. Fate model which relates inventory concentration with in chemical environmental will be developed. USEtox, one of the most advanced methods will be applied in this research.
2. Exposure analysis will be adopted to estimate the amount of exposure caused by the emission of chemical.
3. Effect analysis will be applied to evaluate impacts on ecosystem and human health. Characterization factors reflecting environmental conditions of Japan and Asian countries will be developed through these analyses.

MO 434 LCIA and fate of metal-oxide engineered nanomaterials in freshwater
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Life cycle assessment (LCA) has in only past years proven to be a valuable tool for environmental impact assessment of products and systems, but with the introduction of more new products that contain engineered nanomaterials (ENMs) the tool has experienced an obstacle in terms of performing a holistic life cycle impact assessment (LCIA) of ENMs and the engineered nanoparticles (ENPs) that these materials can release to the ecosystem. Metal-oxide ENMs are often used in consumer nanoproducts where TiO2 and ZnO are considered industry-popular within this group, along with Ag being a widely used ENM metal in consumer goods. Today there is a limited amount of LCA case studies on ENMs published as scientific articles. To be exact these count around 13 studies and in most of those there is a focus on non-toxic impact assessment categories that mainly are related to the raw material and production stages of an ENM product life cycle. Performing a conventional LCIA of an ENM product it can be also concluded that modelling is rather difficult due to missing inventory data and many assumptions that in the end result in cradle-to-gate processes (usually associated to bulk metal production) dominating the impact. The metal-oxide and ENMs are incorporated into a polymer matrix in the performed LCA study. However, release of ENPs to the environment may still occur through, for example, wear and tear. The fate, exposure and effect of ENM by discharge of many of the process affecting the fate, exposure and effect. Fate can be considered a central research area that needs better understanding and is the focus of this study. Fate modelling is performed on metal-oxide ENMs with a focus on the two important processes of aggregation and dissolution. By quantifying fate in freshwater through these two processes there is a more simplified model of the removal of metal-oxide ENPs due to aggregation followed by sedimentation, and the removal of ENPs through dissolution. Thereby concentration in the freshwater compartment can be estimated and used for improving LCIA in the assessment of particle release by linking the metal ENM concentration in freshwater to potential exposure and effects.

MO 435 Moving forward: sustainability and the U.S. cleaning products industry
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Since the Brundtland Commission of the United Nations in 1987 defined sustainable development, the pursuit of sustainability has been a growing movement for all of society, including industries. While the call for sustainable services, products and processes has been heard, the answer to how these should be assessed and reported has not been determined. Over recent years, the American Cleaning Institute® (ACI) has worked to define and describe how the Institute and its members are committed to sustainable environmental, health and socio-economic practices. From a foundation of adopted principles on sustainability, ACI has initiated a program to substantiate actions taken by industry to be more sustainable. In order to be as comprehensive as possible, and to include both ACI’s ingredient supplier and formulator members, an initial evaluation of sustainability metrics across the membership was conducted. From there, attributes including environmental characteristics were chosen for which member companies reported three consecutive years of data. This information is being used for benchmarking and trend assessment purposes. Cleaning product manufacturers and their ingredient suppliers are being faced with a multitude of different demands and requests for data and information that could support development of sustainability reporting and downward the supply chain. ACI’s work is creating a program that supports these demands and demonstrates the contributions of the industry to the goals of sustainable development, while engaging in the development of numerous sustainability programs. Engagement in the sustainability programs of other organizations provides the opportunity to have the industry’s approaches incorporated into their processes. The United States cleaning products industry is working towards sustainability and the development of tools to assess the industry’s achievements. The unique industry approaches for reporting sustainability is still developing, the U.S. cleaning products industry is making substantial progress in reporting major industry-wide trends.

MO 436 Comparing the chemical footprint of hydraulic fracturing fluids used in natural gas extraction
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Hydraulic fracturing, commonly referred to as fracking, is a process by which high pressure fluids are injected into a well to propagate fractures in the rock formation to enable extraction of natural gas and oil from previously untappable sources such as deep shale formations. Although fracking is sometimes used in reference to drilling and completion operations, a more simplifying modern term of hydraulic fracturing. Fracturing fluids used in hydraulic fracturing are essential for unlocking natural gas and oil. Typically, fracture fluids are composed of water, a selected surfactant, or other material, and crosslinked by a polymer. The use of these complex mixtures of fluids and regulatory agencies in the United States are now requiring disclosure. Using composition information available through the public database, fracFocus, we have evaluated the chemical footprint of several fluids to describe the overall potential risk posed by each chemical to the environment and human. The chemical footprint provides an assessment of the environmental performance associated with each chemical and a means for selecting between various ingredients.

MO 437 Application of ERICA index to evaluation of soil ecosystem health according to sustainability threshold for industry impact
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The idea behind ERICA, Borai et al. 2010, was to build a modular and upgradable index. The aim of the improved ERICA is to give an instrument that can be used to measure the environmental effects of engineered substances introduced into the environment. This will be of great help for “green” processes and sustainable industries and may help to advertise their product as safe for the environment following impact assessment not derived only from political and economical chosen limit thresholds values.

In this study, a new theoretical index was used for the soil compartment in assessing sustainable use of new chemicals in the environment. The fact that a certain new compound added to an existing scenario will not pose additional risk is currently not considered by any legislation. Thus, industries able to show this will get an extra value for their products. Furthermore this will lead to a awareness by industries of how much their product are impacting the environment in a realistic way as most possible.

For example degradation is of extreme importance in the soil compartment to understand how the chemical will be transformed and how properties like e.g. persistency and mobility will be different in case of transformation products of the parent compounds. A concise methodology ERICA can be used as a prioritization system, while in case a detailed risk assessment is needed, it is possible to check every step of ERICA procedure and obtain the partial results concerning for example different compartment impact or human health risk assessment or environmental specific target risk assessment.
The paper tries to explore, with examples, the influence of these on decision making of an LCA practitioner. The paper also tries to explore some of the gaps that are usually overlooked in the process.

1. Impact on the LCA practitioner

- Difference in pollutants emitted from LCI versus the characterization factors available in the LCIA methodology
- Need to include LULUC in LCA of forestry products
- Carbon footprint methodology is the most established indicator in the forest industry but still lacks the inclusion of biogenic carbon. The aim is to develop a hydrologic model for the 'green water' in boreal forests and discuss its relevance.
- Land use and biodiversity are gaining more interest but are to be defined more precisely from the forestry basis.
- Water footprint is under rapid development, and new methodologies do not make any specific claims.
- The project will evaluate the existing methodologies and participate in method development in ISO 14046. The aim is also to develop a hydrologic model for the 'green water' in boreal forests and discuss its relevance.

2. Impact categories covered in the methodology

- There is a strong need for methods within life cycle assessment (LCA) that enable the inclusion of all complex aspects related to land use and land use change (LULUC). Here, the aim is to produce biodiversity indicators that are commensurate for each impact category.
- There are different LCIA methodologies developed by various researchers leading to difficulty in selection during an LCA study.
- The methodology is still open and up-to-date scientific views are needed to identify the land use related environmental aspects and to quantify the impacts. Focus is on resource depletion and soil quality impacts of forestry.

3. Life cycle assessment (LCA) method selection: an LCA practitioner’s perspective

- The paper uses examples of both current and historical data, along with new information, to illustrate the importance of considering both time horizons and allocation methods.
- The paper also considers the importance of including all relevant impacts in an LCA study, highlighting the need for methodological consistency.
- The paper concludes with a discussion on the future directions for LCA methodology development.
processes e.g. pasteurization, refrigeration, container material construction etc. Nevertheless, other methods were kept in the framework of the sensitivity analysis. They gave a valuable insight of the drivers that might appear in the results and help in further refining of the interpretation of the result.

MO 444
IMPACT World®: a new global regionalized life cycle impact assessment method
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Most of the impacts modelled in life cycle impact assessment (LCIA) are regional or local. However, LCIA methodologies currently offer generic characterization factors (CFs) that do not account for the spatial variability of impacts. Some LCIA methodologies have partially addressed the issue of regionalization (IMPACT 2002+, ReCiPe, LIME, or LCIA using geographic indicators). However, these methodologies do not account for the spatial variability of each geographic region.

This work presents a new regionalized database, named IMPACT World+, which aims at providing a harmonized database of regionalized impact factors to support life cycle impact assessments at the regional or national level. The database is based on a comprehensive environmental assessment performed on over 500 materials and processes. IMPACT World+ is a new LCIA method which will increase both the relevance and the discriminating power of LCA by allowing to account for uncertainties and spatial variability. Many other improvements have been integrated into the methodology making it more environmentally relevant and scientifically robust.

MO 445
An open source software for Emergy calculation with life cycle inventory databases
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Emergy accounting is a relatively novel environmental accounting method which has gained credit during the last decade, but is still affected by several drawbacks in its calculation procedure and in its general methodology which prevent it from being widely used. This work presents a new open source software specifically created for allowing the rigorous calculation (respecting all the algebra rules) of emergy accounting and thus for the development of regionalized emergy assessment. The software could be potentially used for a consistent emergy calculation of any product in a LCI database and therefore for a complete combination of LCA and Emergy accounting.

MO 446
Complementing ecological footprint with risk analysis for the ecoscience of footwear
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The ecological footprint (EF) was created in the early nineties and has been gaining in popularity ever since. As a pioneering footprint, its applications has expanded and now they are not limited to the regional or national level but include the evaluation of production processes and products. Nevertheless, as a land-based indicator it can hardly account for all of the environmental burdens that may occur during an industrial process or the development of a business activity. Therefore, it is advisable its combined application with complementary environmental evaluation tools.

In this work, this proposes the joint application of EF and environmental risk assessment (ERA) to evaluate footwear design proposals from an environmental point of view. The EF component approach based on life cycle inventory was employed to appraise the environmental impact of the shoe-making process. Thus, individual EFs were calculated for each input/output material and energy flow in the inventory data and then aggregated to estimate the total EF of the pair of shoes. On the other hand, the ERA is a standardized process for the estimation of the magnitude, probability and uncertainty of adverse effects on health derived from the exposure to substances in an environment. In this work, the environmental risk assessment is performed using USEtox model for toxic impacts and water use impacts are included for the first time in an LCIA method.

The results show that the application of EF and ERA helps to allocate priorities and provides more information concerning the environmental impact of footwear products.

RA11P - Guidance documents and guidelines for environmental risk assessment (ERA): needs, developments and progress

MO 449
Mollusc reproductive toxicity tests - Development and validation of test guidelines
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The Organisation for Economic Co-operation and Development is promoting the development and validation of mollusc toxicity tests within its test guidelines programme, eventually aiming for the standardization of mollusc apical toxicity tests. Through collaborative work between academia, industry and stakeholders, this study aims to develop innovative partial life-cycle tests and identify issues in the freshwater gastrotraphs Potamopyrgus antipodarum and Lymnaea stagnalis, which are relevant candidate species for the standardization of mollusc apical toxicity tests assessing reprotoxic effects of chemicals.

Draft standard operating procedures (SOPs) have been designed based upon literature and expert knowledge from project partners. Pre-validation studies have been implemented to validate the proposed test conditions and identify issues in the freshwater gastrotraphs Potamopyrgus antipodarum and Lymnaea stagnalis, which are relevant candidate species for the standardization of mollusc apical toxicity tests assessing reprotoxic effects of chemicals.

The selection of fibres produced under sustainable criteria and the reduction of the material’s consumption was recommended, since the area requirements would be minimized and the absence of hazardous compounds would ensure safety conditions during the use stage.

RA11P - Guidance documents and guidelines for environmental risk assessment (ERA): needs, developments and progress
All laboratories found a concentration-dependent decrease of fecundity in both species under Cd exposure. Values of NOECs and LOECs were generally homogenous controlling exposure concentrations, and possibly BPA leaching from test equipment. This study also highlighted that factors, such as e.g., snail origin, temperature and test chemical stability in water, must be controlled in mollusc toxicity tests to avoid data dispersion which may impede the interpretation of effects. Applicability and limitations of the test proposed for L. stagnalis will be assessed after completion of the on-going pre-validation work. In both cases, optimization of experimental conditions and test procedures is required before the SOPs are ready to be proposed as OECD test guidelines.

MO 450

Temperature effects on reproduction in Potamopyrgus antipodarum - optimizing a standard test protocol with molluscs

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Aquatic ecosystems are exposed to a multitude of chemicals via surface runoff, landfill sites or sewage treatment plants. Many of these substances may affect growth, development and/or reproduction of aquatic species and therefore endanger the survival of populations. Standardized and internationally accepted OECD guidelines for the testing of chemicals are not available for invertebrates with gametes released into the water, which represent the second largest clade. The German Federal Environment Agency (UBA) and the Department for Environment, Food and Rural Affairs of the United Kingdom have supported the preparation of a Detailed Review Paper on Molluscs Life-cycle toxicity testing and a pre-validation round-robins with the parthenogenetic mudsnail Potamopyrgus antipodarum as standard test organism. Currently, the project aims to optimize and define test and breeding conditions to minimize variations of test results in preparation of an international validation exercise.

In this study we investigate reproduction variations at the proposed standard temperature (16°C) and two lower temperatures throughout the year. Therefore, we acclimatized P. antipodarum, L. stagnalis and P. lutea at the three temperatures and tested the species in a parallel life cycle test at the three temperatures. This test consisted in two parts. In the first part, snails were exposed to 1, 3, 10, 30 and 100 µg/L octylphenol at each temperature scenario and at different times of the year. Varying embryo numbers in the brood pouch. For the density experiment, reproduction of snails kept in groups of 5, 10, 20, 40, 80 or 160 specimens per 200 mL DRP medium was investigated after 4 weeks. Subsequently, snail origin, temperature and natural seasonal reproduction patterns can modulate reproductive responses towards a chemical and even mask effects, we assessed sensitivity variations caused by these factors in reproduction tests with the endocrine disruptor octylphenol. To this end P. antipodarum was exposed to 1, 3, 10, 30 and 100 µg/L octylphenol at each temperature scenario and at different times of the year and the resulting effect concentrations were compared.


MO 451

How breeding conditions influence substance effects - the need for a standard test protocol with molluscs

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Up to now effect studies with molluscs play a minor role in regulatory risk assessment of substances because OECD guidelines for the testing of chemicals have not considered this group of invertebrates. To fill this gap, we established a breeding protocol for the two planorbid species Lymnaea stagnalis and Potamopyrgus antipodarum. The protocol includes tests with different breeding media and temperatures and allows to compare different densities after 4 weeks with 12 µg cadmium/L (CAS: 67-68-5) without further density stress for 4 weeks. In the temperature experiment, snails were acclimated at 7 and 25°C for 4 weeks. Then they were additionally exposed to 5, 10, 20 or 40 µg bisphenol A/L (CAS: 80-5-7) at 7, 16 and 25°C. Reproduction was assessed after 4 weeks.

The breeding media resulted in significantly varying embryo numbers of P. antipodarum between all test media after 4 and 8 but not after 12 weeks. Varying snail densities affected reproduction significantly. Furthermore, differing snail densities before the start of the cadmium exposure had a significant impact on the sensitivity towards the heavy metal: the sensitivity increased with density stress in the pre-exposure period. At 7°C and 25°C, a significant increase of reproduction was already observed for the 10 and 20 specimens per 200 mL DRP medium. For P. lutea, the results confirmed temperature as a trigger for reproductive output. In summary, different breeding and test conditions like density or temperature affect the sensitivity towards chemical exposure of P. antipodarum in subsequent reproduction tests. To gain reproducible test results with low variability among laboratories, the definition of standard test conditions is essential.

MO 452

A partial life-cycle toxicity test to assess the effects of endocrine disruptors in the great pond snail Lymnaea stagnalis

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Molluscs are highly sensitive to endocrine disrupting compounds (EDCs) and therefore are of great interest for environmental hazard and risk assessment. The Organisation for Co-operative Economic Development (OECD) is thus promoting optimization and validation of mollusc-based toxicity tests. Standardised test methods for assessing effects of reprotoxics, and hence a number of EDCs, are currently under development in various mollusc species, including the great pond snail Lymnaea stagnalis. Based upon our ongoing work, we developed and performed a partial life-cycle toxicity test with P. antipodarum and L. stagnalis for the ESD of 2011. The test consisted in two parts. In the first part, snails were exposed to the toxicants during 56 days in semi-static conditions, as proposed for the OECD test method under development. Survival, growth and fecundity were regularly monitored. Egg-clutches produced in this part of the test were collected and exposed to clean test water during 35 days, which represented the second part of the test. The biological quality of offspring was assessed through the estimation of the frequency of abnormal eggs in clutches, their mean hatching rates, and the mean shell length and dry weight of hatchlings. Data were analyzed using standard statistical approaches, as recommended by OECD for the analysis of toxicity test data. Preliminary results generated over the last 3 years confirm that L. stagnalis is sensitive enough to highlight the reproductive effects of the selected EDCs, or if the monitoring of offspring performances is also required to significantly detect those effects. Obtained results will thus contribute to the process of optimizing the sensitivity of toxicity test data based upon L. stagnalis for use in environmental hazard and risk assessment.

MO 453

Environmental risk assessment of a disinfectant for milk extraction systems: comparison of 2 emission scenario documents and EUSES

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The environmental risk assessment of a disinfectant for milk extraction systems was performed with 1) EUSES 2.1 based on local consumption estimates extrapolated from European average data and 2) EUSES 2.2 based on Emission Scenarios for Biocides (ESD), published in 2002 and 2011, for local consumption estimates (both inside a dairy farm). The ESD of 2002 describes emission only followed by application to agricultural soil, whereas the ESD of 2011 only describes emission to sewage. Moreover in the ESD of 2011, degradation is not taken into account in Tier 1, whereas in the ESD of 2002 a formula for degradation was already included. Calculations were made for 100% sewage as worst-case scenario. Scenario 2.5.1 of EUSES (MFA, dosed in accommodator) was selected from the exposure scenarios of biocides on a local scale, because there is currently no scenario for PT-3/4 biocides evaluated inclusion in EUSES and because scenario 2.5.1 resembled most the PT-3/4 use. With the ESD of 2002, the RCRA were all below 1, whereas with the ESD of 2011, the RCR was <1 for one active substance, but >1 for the other substance and a refinement of the calculation was performed. FEC values obtained with the Tier 1 ESD 2011 calculations were about 10 times higher than those obtained with the ESD of 2002 and about 100 times higher than with EUSES. This can be explained by the fact that the ESD of 2011 does not take into account degradation. An advantage of the ESD of 2011 is that the scenario is more consistent with current practices and that EUSES could be applied in replacement of the ESD. A more accurate environmental risk assessment would be possible with EUSES when a PT-3/4 scenario becomes available.

MO 454

Can we assess environmental risks of antifouling paint usage with the prevailing BPD? P. Ytreberg, L. Johansson, B. Eklund

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Canopy fouling organisms are designed to leach toxic biocides, it is essential to evaluate, prior to use, their effects on aquatic ecosystems, including toxicity to aquatic organisms. This evaluation process, known as environmental risk assessment (ERA), is used during the authorization of anti-fouling coatings and is regulated in the European Union (EU) by the biocidal product directive (BPD). In the ERA, the predicted environmental concentration (PEC) of biocides, due to leaching from the coating, is determined using the modelling tool MAMPEC. In this model, default scenarios representing EU harbor and an EU marina in incorporated in the software and used in the predictions of biocides. However, these default scenarios may be challenged as they have often been overlooked in ERA of antifouling formulations. Removal of fouling by pressure hosing usually occurs close to the marina, e.g. at a slipway, when the boat is lifted out of the water after the seawater. The fouling that is still present on the hull after pressure hosing is typically being scraped off at the boat yard. In this process also antifouling paint particles (APP) will be removed and deposited on the ground. These cleaning activities are not regulated to any great extent, i.e. leisure boat owners are not obliged to collect the waste and the removed APP. Hence, the boat yard may act as a substantial source of contaminants to the recipient (i.e. the marina) as precipitation infiltrates the soil resulting in leaching.
and transportation of biocides. The aim of the current study was to determine the leaching of biocides from a boat yard allocated in Stockholm archipelago. We designed the experimental part to quantify the annual leaching of biocides from the contaminated soil to the marina. The data were subsequently used in a full-scale ERA of antifouling paint usage by determining the contribution of different biocidal sources to a marinair, from boat hulls, pressure washing and from the soil at the boat yard. The results showed that biocides from pressure washing and soil to be a substantial source of biocides to the marina. These findings suggest that a PEC-value based on only the biocidal release from boat hulls to be inadequate in predicting environmental risks of antifouling paint usage.

MO 455

Ready biodegradability testing for Insoluble and volatile substances
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Under REACH a PBT/PvP assessment is required for substances that are manufactured or imported in amounts of >10 tons per year. The first step of persistency assessments is a screening like the tests on ready and inherent biodegradation provide. Often substances fail the criterion for ready biodegradability or an unequivocal assessment is not possible. The substance is not bioavailable for the degrading bacteria due to its low water solubility and/or volatility. Careful consideration of the physico-chemical properties of a substance is therefore necessary to choose the appropriate test design. As the biodegradation of a substance strongly depends on its mass transfer and the bioavailability for the degrading bacteria, there are several technical issues which need to be addressed while testing the biodegradability of poorly water soluble and/or volatile substances. For this purpose, the OECD guidelines for ready and inherent biodegradable tests (OECD101A-E, OECD110), which differ mainly in several aspects, the ratio test solution/headspace, the agitation and aeration during testing and the type of analysis. Based on the substance properties the test type has to be chosen carefully e.g. the use of the aeration or agitation method can result in losses of the substance and underestimated biodegradation. The influence of the test type on the biodegradation of insoluble and volatile substances was investigated and the results will be presented.

MO 456

OECD Guideline 209: investigation of validity criteria
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The revision of the OECD guideline for the Activated Sludge Respiration Inhibition Test (ASRIT) was finalized in 2010. Within the revision the validity criteria were changed significantly and a new criterion was included. The specific oxygen uptake rate of the activated sludge should not be less than 20 mg oxygen per one gram of activated sludge (dry weight of suspended solids) in an hour. As the oxygen uptake rate of active sludge from many municipal treatment plants shows fluctuations and periodically decreases below 20 mg O2/(g[GREEKX]h), the significance of the validity criterion was examined. It is assumed, that parameter like season, sewage flow and weather conditions influence the oxygen uptake rate. To confirm the assumption, reference tests were conducted over several months and the activated sludge was collected at different days of the week. The dependence of the oxygen uptake rate on the assumed parameter was evaluated. Furthermore the correlation between the oxygen uptake rate and the sensitivity of the activated sludge was examined. The results of these investigations will be presented.

MO 457

Development of interim guidance for the inclusion of non-extractable residues (NER) in the risk assessment of chemicals
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Bound residues (BR), including non-extractable residues (NER), are an important factor in PBT (Persistence, bioaccumulation and toxicity) assessment and risk assessment of chemicals. Precautionary risk assessments usually assume 100% bioavailability, i.e. all of the chemical present, is available for degradation or to have potential toxic effects on the environment and/or its biota. This precautionary approach generally overestimates the exposure concentration by the amount that is not available and therefore overestimates the level of risk to the environment. It is also well documented that chemicals that are irreversibly bound to solids are less degradable and less toxic than the total residue would predict. Currently, there is no agreed guidance on how to determine what is measurable or not and how it should be considered in the risk assessment.

An ECETOC workshop ‘Significance of Bound Residues in Environmental Risk Assessment’ was held on 14-15 October 2009 in Brussels, and produced a framework outlining a possible decision tree for improving the risk assessment of NER, together with the identification of key research needs to address gaps in the current knowledge base.

Subsequently, an ECETOC Taskforce was set up to: i) critically evaluate the proposed risk assessment framework developed following the ECETOC workshop and assess its utility as an interim approach for regulatory assessment of chemicals; ii) develop suitable guidance and trigger values to enable the decision tree to be used and test the utility of the scheme using suitable case studies; and iii) Provide guidance on study design to provide the appropriate quality of data needed for the risk assessment framework to function within a regulatory decision making system.

The progress of this taskforce will be presented, along with recommendations for addressing Bound/non-extractable residues. An example of a risk assessment using this approach will be presented.

MO 458

Granivorous birds - exposure reduction by de-husking behaviour
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With the publication of the current EFSA Guidance document on bird and mammal risk assessment, exposure of granivorous birds to treated seeds has received increased attention. Seed-eaters are considered no longer as indicator species only for seed treatment uses, but also regarding foliage spray applications resulting in exposure through consumption of seeds and kernel as well as via seedling treatment and contamination of the soil. For granivorous mammals several studies have been conducted and data is published demonstrating that de-husking i.e. removing of the husk (including large portions of residues) prior to ingestion, can significantly reduce the exposure in crop seeds (e.g. Brühl et al. 2010, DEFRA research report 2010). For birds it is long and well known that several behavioral skills and traits are common across different bird families to remove the husk or the seed coat (e.g. Ziswiler 1965).

One method to quantify the reduction of such exposure routes is to conduct pen studies, i.e. offering treated seeds to caged individuals and measuring the amount of residues (or marker substance) in the remaining seed husks. Such approaches and test designs are feasible for crop and weed seeds.

Since small granivorous birds are often considered as worst-case indicator species (due to their low body weight) their theoretical exposure is relatively high in default risk assessments. Based on the de-husking behaviour of birds (e.g. finches, sparrows and buntings) we will present data and study design proposals on how to use this behavioural function within a regulatory decision making system.

The first ECETOC technical report and associated workshop presented a science-based concept on how to identify endocrine disrupting properties of chemicals for both plant and animal species. It was recognized that the concept needed further refinement, including specificity. For environmental assessments the lead toxic effect can be considered within a study/species or between different studies/species within an environmental compartment.

A study under an endocrine mediated endpoint can be affected below, at the same, or above the threshold of general toxicity. This should be considered in the first part of the original report. In the second part, the concept applied mechanistic and aipal in vivo studies to decide whether a substance is a potential endocrine concern or not.

A substance should only be considered of high concern when the endocrine mediated effect occurs at concentrations lower than those that cause other significant toxicity.

In the case where the endocrine mediated effect is not the lead effect, i.e. the endocrine effect is observed at a higher concentration than that causing other toxicity, then the substance should be treated as non-endocrine based on the end point.

Once a population-relevant endocrine effect has been confirmed, the next step in the environmental evaluation is to consider the specificity across taxa. Although endocrine effects may be observed as the most sensitive ‘(lead) effect within one study or organism, this effect may be accounted for in a risk assessment by more sensitive non-endocrine effects in other taxonomic groups of safety that sufficiently covers endocrine specific effects. Any risk management measures based on the lead toxic effect in one taxon will also be protective of other toxic effects (including endocrine effects) in other taxa.

Such substances should not be considered as endocrine disruptors, since they do not cause adverse endocrine effects in non target species under realistic conditions of use.

MO 460

Concentration-response relationship for teratogenic effect of 17β-oestradiol in eelpout Zoarces viviparus
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In recent years increased frequencies of malformations among eelpout embryos have been detected in monitoring programs in several Baltic countries (1,2,3). Malformations can be induced in oviparous fish species by exposure to chemicals, including endocrine disrupting substances, in the laboratory. Eelpouts are the only viviparous fish in Northern Europe, which makes them very suitable for investigation of mother-offspring interactions and effects in the offspring upon maternal exposure to various chemicals. The causative agent or agents in organisms examining the underlying malformations in the monitoring programmes are not known, but malformations upon exposure of pregnant eelpouts...
to octylphenol and 17β-estradiol (E2) have been observed (4). To investigate the concentration-response relationship for malformations in eelpout embryos induced by E2, we investigated the embryonic development upon exposure of pregnant eelpouts to (E2) at nominal concentrations between 12.5 and 500 ng E2/l. The experiment was terminated after 40-44 days of exposure. The various types of abnormal development among the embryos (early death, malformations of eyes and spinal cord) were evaluated and the frequencies were recorded for each experimental group. Exposure of female eelpouts to environmentally realistic concentrations, 132.7 ng/l, of 17β-estradiol early in the pregnancy increases the abundance of malformations in the embryos. The study further showed that delaying the onset of exposure to the highest dose of E2 by approximately three weeks reduced the frequency of abnormal larvae, suggesting that timing of the exposure is crucial.


**MO 461**

**Use of field studies in different regulatory frameworks**

E.M.W. de Jong

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The poster discusses the different roles of field studies in the different regulatory frameworks. In the tiered approach followed in the registration procedure of plant protection products (PPP), field studies are frequently used in the higher tier. These higher tier studies are used to generate additional information, in order to lower the safety factors of the lower tiers, and to show recovery after an initial effect. For a number of field study types (e.g. mesocosm studies) detailed guidance is available, as well as guidance for the assessment of the studies. In most other regulatory frameworks (e.g. biocides, pharmaceuticals, GMO’s, REACH) the role of field studies is less clear. At the most limited guidance is available, that is not very specific. The poster discusses the possibilities and the impossibilities to use the guidance for field studies from one framework for another framework.

In some cases the same active substance is assessed in different frameworks (e.g. PPP’s and biocides). In this perspective a number of initiatives have been taken to study whether and how field studies, designed and conducted for PPP registration can be used for biocide registration. As an example the use and assessment of mesosum studies in PPP and biocide registration is elaborated.

**MO 462**

**Experimental design and statistical evaluation of ecotoxicological community studies**

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Ecotoxicological field studies may be conceived by industry notifiers in association with external consultants and contract research organisations (CROs). The results may then be interpreted and summarised before inclusion in a regulatory submission in support of product registration. The submission will be reviewed by representatives from Member State (MS) Competent Authorities, in terry, in the conduct and interpretation of such studies. There is a need, therefore, for guidance on both ecotoxicological field study experimental set-up and statistical analysis of ecotoxicological community data - from aquatic mesocosms (invertebrates, algae and/or higher plants), surface/burial-dwelling non-target arthropod (NTA) field studies and full-field soil mesofauna studies.

Firstly, this project seeks to provide guidance on experimental set-up of ecotoxicological community studies from a statistical point of view, such as replication, plot size, study layout, randomisation. Secondly, the project will provide guidance on the statistical analysis of ecotoxicological community effect data. This will include use and interpretation of both univariate and multivariate methods. The project will summarise existing guidance and use examples available in the literature to illustrate key points.

This work is funded by the UK Chemicals Regulation Directorate.

**MO 463**

**Development of a framework for toxicological and ecotoxicological risk assessment of fertilisers, soil improvers and related products**

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The use of fertilisers, soil improvers and related products may entail toxicological end ecotoxicological risks through the presence of specific chemical substances and/or pathogens. In Belgium, there is currently no real technical guidance on how to conduct a toxicological and ecotoxicological risk assessment of fertilisers, soil improvers and related products. Therefore, an evaluation is made of existing EU methods for toxicological and ecotoxicological risk assessment in similar domains (REACH, plant protection products, biocides, etc[DOTS]) and of existing national approaches of risk assessment of fertilisers, soil improvers and related products in other EU member states. The application and relevance of the identified relevant methods are further evaluated and tested in case studies for specific products. The results demonstrate that screening methods, identifying the most critical human health and environmental endpoints based on the hazards of the constituents, improve the usability and applicability of the selected risk assessment methods.

**MO 464**

**Chemical risk assessment under chemical substances control law in Japan**

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The purpose of Chemical Substances Control Law (CSCL) is to evaluate, before manufacture or import, whether or not new chemical substances have properties such as persistent, bioaccumulative or toxic (PBT) ones, which may cause environmental and human health issues. A risk assessment based on the potential environment and man health risk is required. All involved parties may be experts in the conduct and interpretation of such studies. There is a need, therefore, for guidance on both ecotoxicological field study experimental set-up and statistical analysis of ecotoxicological community data - from aquatic mesocosms (invertebrates, algae and/or higher plants), surface/burial-dwelling non-target arthropod (NTA) field studies and full-field soil mesofauna studies.

Firstly, this project seeks to provide guidance on experimental set-up of ecotoxicological community studies from a statistical point of view, such as replication, plot size, study layout, randomisation. Secondly, the project will provide guidance on the statistical analysis of ecotoxicological community effect data. This will include use and interpretation of both univariate and multivariate methods. The project will summarise existing guidance and use examples available in the literature to illustrate key points.

This work is funded by the UK Chemicals Regulation Directorate.

**MO 465**

**Status quo and challenges in the EU scheme of environmental risk assessment for nanomaterials**

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2Although high potential benefits of engineered nanomaterials (NMs) for overcoming the technological limitations of conventional materials, the unique properties of NMs raise new environmental challenges in understanding, predicting, and managing potential risks. In order to establish a standard risk assessment of NMs, different regulatory frameworks have been suggested in recent years in various fields of chemicals, cosmetics, foods, environmental safety and waste in the EU.

As a representative regulation for controlling the potential risk of NMs manufactured or imported in the EU, REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals) controls the use of NMs. In order to address the NMs’ risk issues, REACH requires screening and testing information. Nevertheless, specific REACH guidance for NMs is not readily available. For developing the draft technical guidelines for risk assessment of NMs, the REACH implementation Projects on NMs (RIP-ONs) have recently been conducted by the EU Commission. However, the draft guidelines are still placed on the unsatisfactory level to reach a consensus on the risk assessment scheme of NMs in accordance with regulatory purposes. The objectives of this study are to analyse and map the status quo and future research challenges in the EU Nano risk assessment scheme based on the RIP-ONs and related recent studies with focusing on environmental risk assessment (ERA). The major challenges in regulating NMs are identified as the wide variety of NMs and applications, the limited knowledge on the toxicity of NMs in living system and their transport in living and environmental systems, the lack of harmonized standards or guidance, and the issues related to classification of NMs.
The nanotechnology industry is rapidly growing, and the production and use of engineered nanoparticles (ENPs) is already a billion euro industry. ENPs are used in a wide range of applications (e.g. consumer goods and plant protection products) which will inevitably result in exposure to the environment. The current study reviews three major European chemical legislative instruments (REACH, the Water Framework Directive and the Cosmetics Regulation) in order evaluate whether the risk assessments under these instruments are suitable for ENPs or whether nano-specific adaptations to these frameworks should be recommended. REACH and the Cosmetic Regulation were chosen since they regulate some of the most important uses of ENPs (e.g. cosmetics and industrial applications). The Water Framework Directive aims at protecting aquatic environments in the European Union, and is thus essential in regard to environmental risk assessments.

The ERA schemes are based on the substantial amount of experimental data that have been generated in the EU funded NanoReTox project (insert project number here). Within the project we have studied the fate and toxicity, to both humans and the environment, of selected metal ENPs. The empirical approach allows us to evaluate the risk assessment frameworks with very specific criteria and thus gives the analysis another dimension compared to an analysis that would have been based solely on theoretical considerations.

MO 465

PBT Assessment: the need for an agreed guidance across persistence - assessment of persistence as an example


The comparison of the two values revealed that the EPM will not necessarily underestimate the risk, even in cases when no additional factor is used. In most cases NOEC-sed is provisionally calculated using the equilibrium partitioning method (EPM), as described in the REACh guidance. The EPM is based on the assumption that both sediment and aquatic organisms are equally sensitive. Only the uptake via the water phase is considered, assuming that substance concentrations in sediment and interstitial water are in a thermodynamic equilibrium. However, notable uptake may also occur via ingestion of sediment or direct contact to sediment. Thus, according to the REACh guidance, the EPM method has to be applied in a modified way for compounds with an octanol/water partitioning coefficient (log Kow) > 5 (or with a corresponding binding behavior, e.g. ionizable substances). In these cases the uptake via ingestion of sediment should be taken into account by increasing the PECsed/PNECsed ratio by a factor of 10.

At which level is an additional factor for EPM within the risk assessment of the benthic environment justified?

MO 466

Stakeholders’ view on ecological risk assessment in the EU: what are we trying to protect and why might we be failing?

A.D. Hunka

A combinatorial approach to safety product regulations is needed to ensure the identification of PBT-substances on a common basis. In this study we gathered information for data on sediment organisms as well as aquatic data and adsorption values from different sources, i.e. EU-RAR, BPD and REACh. From aquatic data and the distribution coefficient, sediment effect values were calculated using the EPM. Hereby we preferred the suspended matter-water partition coefficient (Kpsusp) as the log Kow is only an indirect descriptor for the adsorption on sediments.

In this study we gathered information for data on sediment organisms as well as aquatic data and adsorption values from different sources, i.e. EU-RAR, BPD and REACh. From aquatic data and the distribution coefficient, sediment effect values were calculated using the EPM. Hereby we preferred the suspended matter-water partition coefficient (Kpsusp) as the log Kow is only an indirect descriptor for the adsorption on sediments.

MO 470

Mitigation measures for surface and groundwater based on risk assessment of Plant Protection Products in France: from the risk assessment to the stakeholders

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In Canada, the Canadian Environmental Protection Act (CEPA) 1999 requires that all new substances for use in Canada be evaluated for their potential risks to the Canadian environment and human health while the Food and Drugs Act regulates the sale of human and veterinary drugs. Health Canada, in consultation with representatives from industry, non-governmental organizations and consumer groups has developed environmental assessment frameworks for new substances in products regulated by the Food and Drugs Act. One framework is specific for active pharmaceutical ingredients (APIs) in human and veterinary drugs and has been designed to align with the drug development/approval process. When developing this framework, the starting premise was that the regulatory requirements must be science-based and in proportion to the potential for risk. Furthermore the approach taken was that any new framework should leverage the work done for environmental assessment guidelines in other jurisdictions internationally and harmonize where feasible. This talk will explore similarities and differences between the proposed Canadian Environmental Assessment Regulations and requirements in other jurisdictions with an emphasis on issues that are distinct to the Canadian context.

MO 471

At which level is an additional factor for EPM within the risk assessment of the benthic environment justified?

S. Hofmann

For the different uses assess all PPP may then be labeled with the following phrases, which should be supplemented with specific recommendation regarding the time period or frequency of application (SPe1); and/or specific soil type or situation (SPe2) and/or specific unsprayed buffer zone (SPe3). Since these Standard Phrases for safety precautions do focus on potential risks accounting for both the exposure and the toxicity for various uses of the PPP they are supposed to be suitable indicators since they summarize the frequency of application (SPe1); and/or specific soil type or situation (SPe2) and/or specific unsprayed buffer zone (SPe3). Since these Standard Phrases for safety precautions do focus on potential risks accounting for both the exposure and the toxicity for various uses of the PPP they are supposed to be suitable indicators since they summarize the frequency of application (SPe1); and/or specific soil type or situation (SPe2) and/or specific unsprayed buffer zone (SPe3). Since these Standard Phrases for safety precautions do focus on potential risks accounting for both the exposure and the toxicity for various uses of the PPP they are supposed to be suitable indicators since they summarize the frequency of application (SPe1); and/or specific soil type or situation (SPe2) and/or specific unsprayed buffer zone (SPe3). Since these Standard Phrases for safety precautions do focus on potential risks accounting for both the exposure and the toxicity for various uses of the PPP they are supposed to be suitable indicators since they summarize the frequency of application (SPe1); and/or specific soil type or situation (SPe2) and/or specific unsprayed buffer zone (SPe3).
Information systems on environmental quality standards (EQS) and quality criteria (QC)
might be that different effect assessment methods are used for registration or standard setting. The responsible ministries decided to update the aquatic effect assessment procedures for plant protection products (PPP) in the Netherlands, taking into account the requirements laid down in European legislation with respect to PPP authorisation (Regulation 1107/2009/EC) and the derivation of QS under the Water Framework Directive (2000/60/EC).

In this project, decision trees have been developed for the aquatic risk assessment that can be used in the pre-registration authorisation and post-registration monitoring and evaluation. The core of the approach is that risk assessments are performed at two places in the water system, viz. (1) in edge-of-field surface water and (2) further downstream in surface water that has officially been assigned as WFD water bodies. In our first poster, we summarise the methodology for the edge-of-field assessment and the derivation of QS, taking the information in a regular pesticide dossier as a basis. In this second poster, we present the results of case studies used for the verification of the proposed methodology.

MO 47 - Aquatic effect assessment for plant protection products II. Case studies for the verification of a new procedure in the Netherlands

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A proposal has been made to update the aquatic effect assessment procedures for plant protection products (PPPs) in the Netherlands, taking into account the requirements laid down in European legislation with respect to PPP authorisation (Regulation 1107/2009/EC) and the derivation of QS under the Water Framework Directive (2000/60/EC). The core of the approach is that risk assessments are performed at two sites in the water system, viz. (1) in edge-of-field surface water and (2) further downstream in surface water that has officially been assigned as WFD water bodies. In our first poster, we summarise the methodology for the edge-of-field assessment and the derivation of QS, taking the information in a regular pesticide dossier as a basis. In this second poster, we present the results of case studies that we performed to verify the proposed methodology. For different types of PPP (two insecticides, a herbicide and a fungicide), we performed first and higher tier risk assessments for the edge-of-field on the basis of dossier data and estimated concentrations in water from model calculations for drainage ditches. In addition, we derived QS according to the methodology of the WFD and compared those with measured concentrations on WFD-monitoring sites. On the basis of the results, recommendations for refined the proposed procedures are made.

1 Aquatic effect assessment for plant protection products. I. A Dutch proposal addressing the requirements of the Plant Protection Product Regulation and the Water Framework Directive

MOPC1 - Soil ecotoxicology

MOPC1-1 Linked soil environmental availability and bioavailability of metals to snails: holistic approaches using single chemical extractions and DGT applied to field contaminated soils

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Studies on metal bioavailability in soils are often difficult due to the complex interactions occurring between solid phases, solution chemistry and live organisms. In this work, we assess the bioavailability of Cd, Pb and Zn to the earthworm Eisenia fetida in the presence of a range of soil samples from the field, taking into account various soil parameters which may affect the bioavailability of trace metals. The earthworm species studied is a common model for soil bioavailability studies and has been used as a target organism for the derivation of soil quality standards under the WFD. Our approach consisted in assessing the various available parameters in multiple soil samples and using a combination of soil and environmental-chemical methods to predict the bioavailability. A multiple regression model was developed for Pb and Cd, and the results showed that the use of soil parameters allowed a significant improvement of the prediction (R² up to 0.7). For Cd, the addition of soil characteristics in regression allowed to greatly improve the prediction (R² up to 0.72) although they were not correlated and the addition of soil parameters in regressions did not improve the fit, testifying to the ability of the DGT to properly predict Cd transfer. Considering Cd fluxes, the distribution of Cd in the soil must be known to assess the fate of the compound in soil. For example, NER from pesticides are considered to consist of adsorbed and sequestered parent compounds and metabolites as well as dissolved residues. However, they may also partly derive from bacterial biomass, resulting in harmless bioaccumulative residues. In addition, the formation of soil organic matter (SOM) or humic compounds has long been a dominating topic in soil science because the amount and composition of SOM determines soil quality. The use of eco-toxicological procedures for the assessment of metal bioavailability in soil samples treated with different SOM is still not well defined. Metal immobilization in SOM can hide the presence of soluble fractions of metals which have a potential toxicity to organisms. Therefore, the use of SOM as a soil parameter for metal bioavailability assessment can significantly improve the accuracy of the predictions. The results indicate that a high percentage of the biomass-derived carbon remains in soil, mainly in the non-living part of SOM after extended incubation. The results provide a simple explanation for the high persistence of the organic carbon pool in soil and the importance of SOM in the long-term stability of metal bioavailability. This distribution must be known to assess the fate of the compound in soil. For example, NER from pesticides are considered to consist of adsorbed and sequestered parent compounds and metabolites as well as dissolved residues. However, they may also partly derive from bacterial biomass, resulting in harmless bioaccumulative residues. In addition, the formation of soil organic matter (SOM) or humic compounds has long been a dominating topic in soil science because the amount and composition of SOM determines soil quality. The use of eco-toxicological procedures for the assessment of metal bioavailability in soil samples treated with different SOM is still not well defined. Metal immobilization in SOM can hide the presence of soluble fractions of metals which have a potential toxicity to organisms. Therefore, the use of SOM as a soil parameter for metal bioavailability assessment can significantly improve the accuracy of the predictions. The results indicate that a high percentage of the biomass-derived carbon remains in soil, mainly in the non-living part of SOM after extended incubation. The results provide a simple explanation for the high persistence of the organic carbon pool in soil and the importance of SOM in the long-term stability of metal bioavailability. The present study investigated adverse effects caused by trophic transfer of gold nanoparticles in a soil food chain. The present study investigated adverse effects caused by trophic transfer of gold nanoparticles in a soil food chain. The present study investigated adverse effects caused by trophic transfer of gold nanoparticles in a soil food chain. The present study investigated adverse effects caused by trophic transfer of gold nanoparticles in a soil food chain.
North-West University, Potchefstroom, South Africa
DNA barcoding was used to investigate the phylogenetic delimitations of the major earthworm populations used in ecotoxicological research in South Africa. A total of four focal populations (used in published works) and two non-focal populations were assessed. Three of the focal populations (two laboratory based and one field) are referred to as species in the literature. The last focal population (a field based) has been occasioned as a source of Deroceras reticulatum specimens. Phylogenetic analyses in the COI gene revealed that all the Eisenia populations had been misidentified as all the generated sequences grouped unequivocally with published sequences of E. andrei. Moreover, very high sequence divergence (> 25% K2P) found within E. andrei could signal the occurrence of hitherto non-described cryptic species. The last focal population was found to be a mix colony harbouring D. rubidus, M. micromeris and at least two other unidentified species. These findings are discussed with an emphasis on their implications on the current use of earthworms in ecotoxicological bioassays. Left unaddressed, this situation could globally become a considerable setback to ecotoxicological testing as the present study might not be an isolated case.

MOPC1-6
What is the most relevant earthworm species for testing pesticide effects? A meta-analysis
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Human land use, through intensification of agricultural practices and particularly soil pollution by chemical inputs, has often resulted in loss of biodiversity. Earthworms represent one of the most interesting organisms in soil because they perform important agro-ecological functions since they influence organic matter dynamics and soil structure. They are considered as indicators of soil biodiversity and quality. Pesticide impacts on earthworms are difficult to measure under field conditions due to confounding effects. Ecotoxicological risks are assessed mainly under laboratory conditions. However, the Earthworm E. fetida, is used in many biotests as an authorized model for biological models for these tests. It is also often used in laboratory studies thanks to its short generation time and to its easy culturing. However, there is a controversy about the sensitivity of this species towards pesticides, when compared to other species naturally present in agrosystems where chemicals are used. Using a meta-analysis, we address the following questions: Is E. fetida less sensitive to pesticides than other species? Are earthworms belonging to different genus and ecotoxicological groups differently influenced by pesticides due to their different behavior? What is the relevance of using only mortality as indicator in such studies and tests? Results will allow us giving recommendations for testing effect of pesticides on earthworms in a relevant way. We will also pinpoint the gaps we have to fill in order to understand how pesticides influence earthworm population dynamics in agricultural fields.

MOPC1-7
Soil threats in the Netherlands
Alterra, Wageningen, Nederland
Contamination in the Czech Republic.

MOPC1-8
Monitoring of the agricultural soils
Central Institute for Supervising and Testing in Agriculture (ÚKÚZ), Brno, Czech Republic
Monitoring of agricultural soils in the Czech Republic is monitored by the Central Institute for Supervising and Testing in Agriculture (ÚKÚZ) via three programmes. These are “Monitoring of the agricultural soils”, “Monitoring of inputs into the soil” and an administration of the database “Registry of contaminated sites”.

MOPC4-1
Combined effects of estrogen receptor antagonists on in vitro vitellogenesis
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Several environmental compounds are known to have anti-estrogenic activity and their modes of action (MoA) are believed to include competitive inhibition of 17β-estradiol (E2) binding to the estrogen receptor (ER) or interference with ER-dependent processes through different pathways including increased metabolism of E2 and receptor cross-talk. Concentrations of single substances in the environment are generally low and effects rarely observed. Presence of multiple compounds having the same mode of action may cause concern, however, as their effects may be additive. Additive effects of mixtures can be modelled by the two prediction models; concentration addition (CA) and independent action (IA). The objective of the present study was to determine if the combined effects of mixtures of ER antagonists on the ER-mediated production of the estrogenic biomarker vitellogenin (Vtg) in rainbow trout (Oncorhynchus mykiss) hepatocytes could be predicted by the CA and IA prediction models. Model anti-estrogens (4-hydroxy tamoxifen and ZM 189,154) and environmentally relevant compounds (PCBs and PAHs) were tested to ensure inclusion of compounds from different chemical classes with more or less well known mechanisms of anti-estrogenicity. All eleven tested compounds had the ability to inhibit E2-induced production of Vtg. The potency of the tested compounds differed by four orders of magnitude based on the concentrations for 50% inhibition (IC50). The observed order of potency was 2,3,7,8-tetrachlordibenzo-p-dioxin > 4-hydroxy tamoxifen > 3,3’,4,4’,5-Pentachlorobiphenyl > benzo(k)fluoranthene > 3,3’,4,4’-tetrachlorobiphenyl > 2,3,7,8-Hexachloro-PCB > 3,3’,4,4’-tetrachlorobiphenyl > benzo(a)pyrene > benzo(a)anthracene > 4-hydroxy tamoxifen > ZM 189,154 > indeno[1,2,3-cd]pyrene > benzo(b)fluoranthene > benzo(a)pyrene > benzo(a)anthracene. All tested mixtures inhibited E2-induced production of Vtg in a concentration-dependent manner. The mixtures consisting of compounds with similar MoA were well predicted by the models. The mixture consisting of compounds with different MoA caused deviation from the prediction models consistent with more than additive effects. The results from this study show that CA and IA prediction models can be used to assess combined effects of mixtures of estrogen antagonists with similar MoA. The rationale for the deviations observed with the mixture of compounds with different MoA warrants additional studies to determine the effects of the interactions occurring and to assess potential impacts on the health of organisms.

MOPC2- Endocrine disrupting chemicals: recent developments
MOPC2-1
Dependence between models and tests in the assessment of the endocrine disruption potential of chemical substances
T. Aldenberg, M. Nendza, A. Wenzel
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MOPC2-2
Testing thyroid disruption: which data are available in different regulations to support an initial suspicion for the environment?
T.J. Jüffrenholz, A.K. Klein, C.N., Neubert
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Identification, assessment and regulation of substances having an impact on the endocrine system of ecotoxicologically relevant organisms are of special interest in several legislations in the EU (Chemicals: REACH (EC) No. 1907/2006, Plant protection products: 1107/2009 EEC, Pharmaceuticals: 2001/83/EG (2009) and 2001/82/EG (2009)). Biological activities (w/o070076 ), . Especially, since for this group a hazard-based approach is proposed by all of the legislations. However, identifying potential endocrine disruptors (EDs) might be pretty difficult as special tests systems for EDs are not well established in ecotoxicology and still under development. Therefore, the currently requested test systems are not prepared to identify endocrine disrupting compounds alone and correlate them to adverse effects. During the last years research effort was mainly focused on sexual endocrine disrupting chemicals, inter alia also influencing effects on fish. Nevertheless, effects on the thyroid system were also investigated but are not part of included procedures in ecotoxicology, yet. Thyroid hormones play an essential role regarding development processes in mammals, birds, amphibians or fish. Therefore, influences on the thyroid system might result in adverse effects comparable to effects on the sexual endocrine system given that an initial suspicion for effects on the thyroid system can be derived from standard ecotoxicity test data, more advanced and specialized test systems could be requested (special concern is to be proved in most regulations). But when is a substance expected to have an effect on the thyroid system? And based on which data? And what to do if ecotoxicological studies are lacking completely? Do available non-clinical toxicological data and results of in-vitro test help to decide whether a compound affects the thyroid system? This study aims at summarizing existing data and investigating the suitability of these data which affect the thyroid system. Exemplified data sets of different regulations were compared. Studies and endpoints were investigated and (if available) compared with results of tests on the thyroid system. Additionally,
This presentation aims at comparing relevant systemic toxicity endpoints with endocrine mediated endpoints and pointing out the regulatory important conclusions. Inducible in females and fry by androgen exposure. Issues with using mosquitofish as a bioindicator include the lack of knowledge of the mechanism of anal fin elongation. A consideration of the endocrine disrupting properties could lead to NOEC values as low as 1µg/L. For example with the following species: Crassostrea gigas NOECsperm and will be illustrated using case studies from the University of California Center for Environmental Implications of Nanotechnology. These include: (i) estimation of toxicity in water samples by effect-directed analysis (EDA) to identify endocrine active single substances which are currently not being measured. The results from the whole Tox-Box will be presented. In the second step of this module endocrine active single substances will be used in the H295r-Assay to investigate the mode of action of the toxicity of drinking water. As one part of this project the Institute for Environmental Research at RWTH Aachen University will analyze and establish endocrine activity of drinking water samples. The samples will be provided by Rheinenergie AG as a water supplier and the toxicity of drinking water will be determined. Female mosquitofish were exposed to a vehicle control or one of three doses of the androgen 17β-trenbolone (TB) for 3, 7, 14, or 21 days. Body morphometrics were assessed and livers and anal fins were removed for quantitative polymerase chain reaction (qPCR). In situ hybridization (ISH) was used to evaluate Shh gene expression in the anal fin. This study demonstrates that TB can induce anal fin growth at early time points. One-way ANOVA, p<0.05). This may be due to the ability of a higher dose of androgen to stop elongation and promote the formation of advanced structures (hooks and serrae), MxS was increased in all TB doses at days 7 and Fgfr1 was increased at days 3 and 7 by 10 µg/L TB (one-way ANOVA, p<0.05). Shh was strongly expressed in the distal tip of the anal fin and the result demonstrates 1 µg/L TB had reduced hepatic vtg expression (one-way ANOVA, p<0.05). Future experiments will determine how TB affects anal fin elongation versus differentiation, evaluating MsxC and Fgfr1 expression at additional early time points, and developing an assay to measure tryptophan during TB exposure to compare these data with vtg gene expression levels. Knowledge gained from these and future experiments will be a starting point for future research towards establishing biomarkers of androgen exposure and reproductive health. Nevertheless, there is an ongoing discussion in the scientific and regulatory communities about the severity of the endocrine disrupting properties in comparison with the established toxicological properties of standard endpoints. For the REACH assessment the toxicological properties were judged using the endpoints Adult Daphnia 21 d NOEC of 0.33 µg/L. Even if we would not consider endocrine disrupting properties in our assessment under REACH algal biomass is no valid endpoint (see ECHA Guidance document: R7b R7.8.3.5) and therefore PNEC derivation would have to change. A different lowest NOEC would have to be used: Mysidopsis bahia NOEC 3.9 µg/L. A consideration of the endocrine disrupting properties could lead to NOEC values as low as 1µg/L. For example with the following species: Grassostrea gigas NOEC sperm motility <1µg/L and Arbracia lixula NOECdev abnormalities <0.93 µg/L. This presentation aims at comparing relevant systemic toxicity endpoints with endocrine mediated endpoints and pointing out the regulatory important conclusions. Assessing the developmental toxicology of water samples by effect-directed analysis (EDA) to identify endocrine active single substances which are currently not being measured. The results from the whole Tox-Box project will be used to establish a new guideline regarding the risk assessment of anthropogenic micropollutants in drinking water. The development of quantitative weighting factors for use in a weight of evidence framework for the evaluation of endocrine activity E.M. Mihalci, C. J. Borgert, L.S. Ortego, K.S. Bentley, C.M. Holmes, S.L. Levine, M.S. Marty, B.H. Neal, R.A. Becker Environmental & Regulatory Resources, Durham, nc, United States of America. Applying Pharmaceuticals & Toxicology Inc, Newton, U.S., United States of America. Bayer CropScience, Research triangle park, nc, United States of America. DuPont Crop Protection, Newark, de, United States of America. BASF Corporation, Research triangle park, nc, United States of America. Monsanto Company, St. Louis, mo, United States of America. The Dow Chemical Company, Midland, mi, United States of America. Exponent, Alexandria, va, United States of America. American Chemistry Council, Washington, dc, United States of America. We previously developed a conceptual framework for conducting weight of evidence (WoE) evaluations of endocrine screening and testing data (Borgert et al., 2011). The framework was based on the idea that endocrine toxicity is multifaceted, but with an emphasis on data emerging from the US EPA's Endocrine Disruptor Screening Program (EDSP), and specifically tailored to accommodate endpoints measured in the Tier 1 Endocrine Screening Battery (ESB) assays. The framework calls for a transparent process whereby specific hypotheses are formulated and data quality is evaluated with respect to overarching scientific principles, including primary validity of the measurements, reliability of data reporting, and probative capability of the study design to evaluate causation. The framework also calls for assigning quantitative weighting factors to assay endpoints (WoE) that reflect their relevance for deciding specific hypotheses. This is an important step in the WoE framework as it explicitly recognizes the fact that some assays are very specific and sensitive for evaluating certain endpoints related to endocrine activity but may be irrelevant for evaluating others. For example, an increase in thyroid follicular hyperplasia, and a positive test in the amphibian metamorphosis assay, would be expected to have a very high WoE for evaluating the hypothesis that a chemical has potential thyroid antagonist activity, but would have a very low WoE for evaluating the hypothesis that a substance has potential estrogen or androgen agonist or antagonist activity. The framework recommends that WoE values be derived as numerical weighting factors based on the predictive value of the endpoint for actual endocrine activity in vivo. They are intended to be useful in determining the relevance and developmental toxicity tests, i.e., EDSP Tier 2 tests. This predictive value, however, may be difficult to evaluate until results for a test set of chemicals are available from the Tier 1 ESB and can be compared to results of Tier 2 tests. As an interim step, however, provisional WoE values can be derived based on a rank ordering of relevance for each hypothesis. This rank ordering should also be based on data, but will necessarily involve considerations of mechanism. An example dependency rank ordering of WoE values for evaluating Tier 1 EDSP hypotheses is presented, with particular emphasis on endpoints measured in the fish screening and frog metamorphosis assays.
(iv) taking account of sub-organisinal information and environmental physico-chemical processes in modeling the influence of CdSe quantum dots on bacterial population dynamics; (v) models to predict the results of experiments on the effects of citrate coated silver nanoparticles on phytoplankton-zooplankton interactions. Our approach complements much work on adverse outcome pathways (AOPs) by extending the pathways to include ecological interactions without compromising the critical connections with suborganisinal MOPC3 - Non-target analysis and identification of toxicologically significant emerging pollutants

MOPC3-1
How to evaluate pollution, degradation and metabolisation of multi-families of pharmaceuticals and endocrine disruptors in multiple compartments of the surface water media?

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Evaluations like risks or effects assessments of the presence of various xenobiotics in the environment show a continuously growing interest since the past decades. To evaluate models for degradation of a family of pharmaceuticals and thus impact their use on the environment, scientists need good analytical methods to reinforce data. While literature focuses on single matrices or single family, this work aims to develop an integrative approach of the surface water media; water, sediments and biota. Those methods are combined to relatively simple and short time-consuming extraction techniques investigated for various matrices for the 20 various pharmaceuticals and 28 Endocrine Disruptor.

Natural LC-MS/MS method has already been applied to a benthic model exposed for eco-toxicological study [1]. All presented LC-MS/MS, automated online SPE-LC-MS/MS and nanoLC-MS/MS methods have been validated following ICH recommendations. They allow rapid quantification of multiple pharmaceuticals and endocrine disruptors at environmental levels and have been for the study of real ecosystems in French rivers. In parallel thanks to passive samplers calibrated in the laboratory and validated in situ, huge areas were studied averaged concentration can be determined in parallel on the same field. The quantification of accumulated and metabolised compounds in biota but also degradation products and coefficient partitioning between water and sediments. Those studies even could correlate effects to focused on benthic models. Further investigations with TOP spectrometers should allow non-targeted analysis for identification of new by-products, degradation products or metabolites.

Although this analytical challenge is needed for risk and effects assessments on environment and populations, it is really time-consuming for the analyst. Prioritisation by-products, degradation products or metabolites is crucial to select representative pollutants to help scientists building models. This project opens the discussion for assessments of multi-families of various ubiquitous pollutants with a relatively large panel of analysis for different matrices of the same water surface media.

References

MOPC3-2
Non-target analysis of emerging contaminants - harnessing antibody selectivity

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The coupling of immunoassays to liquid chromatography is described. Fractions of complex environmental samples obtained by RP-HPLC fractionation have been analyzed with immunoassays for carbamasame and sulfamethoxazole. Two metabolites, one conjugate and two structurally not so closely related compounds have been identified by this method. Procedures and limitations are described in the presentation.

MOPC3-3
Development of a water toxicity sensor based on genetically modified bacteria

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The detection and monitoring of pollutants in surface water is routinely done by chemical analysis, biomonitoring using aquatic organisms, or in vitro toxicity assays. Chemical analysis is sensitive and can be fast, but only compounds searched for are detected. Biomonitoring with algae, Daphnia and mussels are excellent for the detection of the total toxicity of for example heavy metals or pesticides. However, not all toxicants with relevant effects can be measured, for example genotoxicants or endocrine disruptors. Laboratory based assays on the other hand are used to perform and can be used on-line in the field.

To be able to fill this gap, a toxicity sensor has been developed, which employs genetically modified Escherichia coli that emit luminescence when they are exposed to compounds with a certain effect. The effect that can be measured depends on the modification of the bacteria. The strain used in the present sensor at the moment responds to DNA damage. The bacteria are immobilized in a sol-gel on a glass slide and an optical fiber which were continuously exposed to a water flow. The goal is to develop a sensor that can be used on-line in the field with minimal maintenance.

The first experiments with the sensor were performed with Dutch tap water, which does not contain added chlorine. The immobilization in sol-gel did not affect the response of the bacteria. The luminescence of the bacteria increased after exposure to nalidixic acid and mitomycin C. The response started approximately one hour after the start of the addition of the positive control.

The concentration of added growth medium had a strong influence on the response and was thereby the most important parameter. A concentration of 2% of Lysogeny broth (LB) was enough to sustain the signal without causing strong fluctuations. Both the background signal and the response of the bacteria declined with time. One possible reason for this might be competition with other microorganisms in the water, but this is still under investigation. As expected, the fiber yielded a signal that was 100-200 times lower than the signal of the plate. This was the result of the much smaller surface area of the fiber tip.

MOPC3-4
Can solar irradiation transform human metabolites of pharmaceuticals back to their parent compound? Insights on the direct photolysis processes and pathways of sulfamethoxazole human metabolites

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Sulfamethoxazole (SMX) is a widely used antibiotic, only partially removed by wastewater treatment and therefore often detected in wastewater effluent and in surface water. How much of the human body load is comprised in the human body into various metabolites, some of which retain certain biological activity, and which have also been observed in the aquatic environment. Moreover the back transformation of certain metabolites to the parent compound has also been reported in certain environments. Both direct and indirect photodegradation processes have been reported to be important removal mechanisms of sulfamethoxazole in the environment; however no research has been conducted on the photolysis of SMX metabolites.

This study investigates the direct photolysis kinetics, quantum yields and products of a selection of SMX metabolites, namely: acetyl SMX, nitro-SMX, nitroso-SMX and hydroxy-SMX. Photo-experiments were conducted in a merry-go-round photoreactor equipped with 300 nm mercury lamps with variable concentrations (10-100um) of SMX metabolites, and analysed via HPLC-UV vis and high-resolution mass spectrometry. Results revealed slower direct photolysis kinetics and quantum yields that were 10-10-fold smaller than those of the parent compound SMX. The photoproducts generated from direct photolysis of SMX and metabolites were identified. The structural similarity of SMX and acetyl-SMX photoproducts point towards the same photolysis cleavage sites. Moreover, the back-transformation of nitro-SMX to the parent compound was observed. These results show that pharmaceutical metabolites can be more recalcitrant to photodegradation and even be a source of pharmaceuticals to the environment.

MOPC3-5
Using mutagenic fingerprints for a compound identification

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One of the major challenges in the field of the environmental chemistry and ecotoxicology is the identification of compounds or compound mixtures which are responsible for adverse biological effects. One major tool for the elucidation of cause-effect relationships is the effect directed analysis (EIDA) which combines the fractionation of a complex sample by HPLC or SPE with the Ames-fluctuation assay, this highly successful approach. One is its complexity and time demand. A biological test strategy which directly provides information about the chemical nature of bioactive compounds or compound classes would complement the classical EIDA because it facilitates a more target-oriented and thus efficient sample fractionation and/or chemical analysis. In the present study such a methodology is developed for the identification of mutagenic compound classes in environmental samples by the Ames-fluctuation assay.

It is well known that many mutagenic compounds are bio-activated by the metabolism of xenobiotics. In recent years different bacterial strains were generated which overexpress one or more enzymes which are involved in the metabolic toxification and/or detoxification of genotoxicants. If a potentially mutagenic compound is bioactive in a given test strain, a modified bacterial strain with different enzymatic equipments. In order to challenge the working hypothesis that such fingerprints allow the identification of compounds or metabolite classes, dose-response-relationships for model compounds were aggregated in a single value, the concentration dependent number of revertants (cdr). The combination of all strain dependent cdr-values for a model compound which represents a chemical class can be treated as a vector which describes the metabolic activability of a compound class. It is tested, if compound classes can be identified by a comparison of these vectors which is based on the calculation of the cosine similarity.

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Innovative sample preparation steps for the analyses of pharmaceuticals in soil
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Chemical products are more and more used for agriculture and domestic activities and are responsible for the spread of several substances in the environment, which can be harmful for animals or humans. Among these products, hormonal steroids and pharmaceutical compounds are of growing concern. If several analytical methods are available to determine the content of these compounds in aquatic environment, few methods were described to allow their analysis in solid matrices. However, the few available data concern synthetic hormonal steroids in soil reveal contaminations which can reach hundreds of ng/kg.

Consequently, the aim of this study was to develop an analytical method for the analysis of traces of 11 hormonal steroids, 16 veterinary or human drugs and 11 other well-known human contaminants, used in soil as pollution tag, in soil. An analytical method both selective and sensitive based on liquid chromatography-tandem mass spectrometry was developed. The optimization allowed the determination of the best conditions for the separation by chromatography and for the detection by mass spectrometry.

The analysis of complex matrix such as soil needed a rigorous sample preparation to obtain a repeatable and enough sensitive analysis to achieve the detection limits required. For the validation step using the nMRChERS (Quick, Easy, Cheap, Effective, Rugged and SafeQuick, Easy, Cheap, Effective, Rugged and Safe) method was set up. This is a relatively new technique, simple, quick and cheap. The use of this method for environmental matrices such as soil is particularly innovative. This technique is based on a liquid-liquid extraction with an organic solvent in presence of salts and several buffers. Several parameters were optimized (sample weight, solvent nature, water and solvent volumes, buffer type). A further purification step was necessary to reduce the matrix effects, therefore the SAX (Strong Anion Exchange) cartridge was used in tandem with the Strata X cartridge for the clean-up.

This analytical procedure allows the treatment of the target analytes in the ng/kg range. After the development of this analytical method, it was applied to real samples of soil collected in several areas of France that were treated differently. Some of them were contaminated with manure spreading, others with sludge spreading and others with atmospheric fallout. Results indicate that all the samples contained several contaminants at level lower than the ng/kg.

NORMAN MassBank - a community driven open mass access spectral database for the identification of organic environmental contaminants
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2Eawag - Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland

The Natural Organic Matter Network (NORMAN) database is a community driven database containing well-known human contaminants, used in this study as pollution tag, in soil. An analytical method both selective and sensitive based on liquid chromatography-tandem mass spectrometry was developed. The optimization allowed the determination of the best conditions for the separation by chromatography and for the detection by mass spectrometry.

The combination of training on the ecoinvent version 3 software by IAI employees, the possibility to directly submit data to ecoinvent, and the capability to create updated datasets by changing only relevant flows will enable the IAI to create updated versions of the datasets as often as it sees fit. One constraint is that the IAI is not able to produce the input data itself and must rely on its members to provide this information.

The wide dissemination is ensured by the fact that (1) the chosen seed database (ecoinvent) is widely used and integrated in most LCA software and (2) the data can be transposed to other existing formats through the use of standard exchange formats. To provide the technical possibilities for this concept, a web-based Open source IT infrastructure is developed, including data storage for the unit modules and a calculation tool for LCA modelling.

The combination of training on the ecoinvent 3 software by IAI employees, the possibility to directly submit data to ecoinvent, and the capability to create updated datasets by changing only relevant flows will enable the IAI to create updated versions of the datasets as often as it sees fit. One constraint is that the IAI is not able to produce the input data itself and must rely on its members to provide this information.

The wide dissemination is ensured by the fact that (1) the chosen seed database (ecoinvent) is widely used and integrated in most LCA software and (2) the data can be transposed to other existing formats through the use of standard exchange formats. To provide the technical possibilities for this concept, a web-based Open source IT infrastructure is developed, including data storage for the unit modules and a calculation tool for LCA modelling.
PestLCI 2.0 is an updated and expanded version of the PestLCI model (Birkved & Hauschild, 2006). The model has been expanded with a fate module for macropore flow, of a chemical emitted to air and the fraction emitted to soil in the case of sludge amendment to soil. SS effluents are nation specific. In order to reflect the national specificity of SSs configurations, statistical data on the SS configurations in all EU countries were compiled from this poster presents a novel approach for quantification of chemical-specific emissions via SSs. SS configurations and the fraction of a chemical with WDU being emitted via receiving the sludge are quantified applying PestLCI 2.0. Groups are modeled with and without sludge amendment to soil. In the scenarios with sludge amendment to soil, the fractions emitted to the environment from the fields GHG-emissions within a Life Cycle Assessment (LCA) are based on generic emission factors (e.g. IPCC factors) only, leaving behind all other site specific conditions [3-4]. Ambitious goals have been set to reduce by 40% the German greenhouse gas (GHG) emissions by the year 2020 [2020 levels). One of the strategies followed by Germany to achieve this target has been the development, use and promotion of renewable energies. Biogas production is a type of renewable energy that has been growing significantly in Germany over the past decades, being the major share in the waste biomass sector specific emissions factors for ammonia, nitrous oxide and methane that can be included in regional-specific LCA studies for energy crops. These emission factors will be derived from direct field measurements of these gases after the application of digestate as well as mineral fertilizer. Furthermore, the project strives to develop precise but also generalisable statements about ammonia volatilisation and alteration of the soil carbon content due to returning digestate from biogas plants to the field. The outcomes of the joint research project should lead to an improved environmental assessment of energy crop production including agricultural aspects such as fertiliser use efficiency and environmental impact of the fertiliser. The project results should provide approaches - not just - for the sustainable production of energy crops but also for a more efficient cultivation of other renewables. References 1. Weiland, P. (2010). Applied Microbiology and Biotechnology 85: 949-860. 2. Heller, M., et al. (2003). Biomas and Bioenergy 25: 147-165. 3. Izart, A., et al. (2010). Journal of Cleaner Production 18: 336-345. 4. C. Pirilli, L. Maiorino 1, M. Pirilli 1, L. Schebek 2, R.R. Sallaberry 1, M. Birkved 2, M.Z. Hauschild 2, A. Werner 2, J. Augustin 2, M. Weisend 3, R. R. Sallaberry 1, T.U. Damrath, Darmstadt, Germany 1, M. Pirilli 1, L. Schebek 2, R.R. Sallaberry 1, M. Birkved 2, M.Z. Hauschild 2, A. Werner 2, J. Augustin 2, M. Weisend 3, R. R. Sallaberry 1, T.U. Damrath, Darmstadt, Germany 1, M. Pirilli 1, L. Schebek 2, R.R. Sallaberry 1, M. Birkved 2, M.Z. Hauschild 2, A. Werner 2, J. Augustin 2, M. Weisend 3, R. R. Sallaberry 1, T.U. Damrath, Darmstadt, Germany 1, M. Pirilli 1, L. Schebek 2, R.R. Sallaberry 1, M. Birkved 2, M.Z. Hauschild 2, A. Werner 2, J. Augustin 2, M. Weisend 3, R. R. Sallaberry 1, T.U. Damrath, Darmstadt, Germany 1, M. Pirilli 1, L. Schebek 2, R.R. Sallaberry 1, M. Birkved 2, M.Z. Hauschild 2, A. Werner 2, J. Augustin 2, M. Weisend 3, R. R. Sallaberry 1, T.U. Damrath, Darmstadt, Germany 1, M. Pirilli 1, L. Schebek 2, R.R. Sallaberry 1, M. Birkved 2, M.Z. Hauschild 2, A. Werner 2, J. Augustin 2, M. Weisend 3, R. R. Sallaberry 1, T.U. Damrath, Darmstadt, Germany 1, M. Pirilli 1, L. Schebek 2, R.R. Sallaberry 1, M. 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SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting

ISPA in intends to update and develop that project in a way to be in compliance with the European Platform on LCA (EPLCA). In this context, the objective of this paper is to illustrate the appropriate methodological approach on the conformity of the data set through criteria (quality, method, nomenclature, etc...) in order to be in compliance with the format defined by European Platform on LCA. Furthermore, ISPA is now planning out a research design aimed at defining the specific site LCI (Life Cycle Inventory) modules concerning the national transport sector based on primary data already available.

Through the acquisition of a methodological approach has been possible to identify the criteria that give the data inventory characteristics of validity and conformity to standards set by the European Platform of LCA, namely quality, consistency and robustness of data. This methodological approach was applied to the modules contained in the database LCA v. 2.0. This LCI database, currently under revision and updated enclosing about 400 manufacturing processes and services, is divided into four main areas (materials and processes, energy, transport and end of life). The verification of conformity was carried out on some modules (related to the energy sector) that previous were transcoded for I-LCA v.3.0 according the format developed by the European Platform on LCA. This first internal review has showed that some of the modules are in conformity with the reference standard, while others modules should be implemented in order to achieve the same degree of conformity.

The creation of a public database LCA, with quality and consistent data, methods and assessments, is extremely important not only for the crucial help in implementing LCA studies, but also in order to promote a different orientation patterns in consumption and production. In this context, ISPA intends to plan out a research design aimed at developing experimental application to a specific transport sector based on primary data already available.

MOPC4-8
Setting up collaborative research on LCA and related topics
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According to the ecosystem compartments where resources flow and stock, we classify the footprint indicators into four patterns, the biologic, the atmospheric, the aquatic (area, volume, weight, etc.). In this sense, the "Footprint Family" would rephrase as a suite of indicators able to measure the environmental impacts from different angles, by linking the release in the environment with the carrying capacity of the system (vulnerability analysis, exposed ecosystem assessment, Eco epidemiology). Thereby, we provide for the first time a definition of the "Footprint" as an approach to convert the environmental impacts of human activities into a physical quantity (area, volume, weight, etc.). In this sense, the "Footprint Family" would rephrase as a suite of indicators able to measure the environmental impacts from different angles, by representing how many resources for each kind human have appropriated. According to the ecosystem compartments where resources flow and stock, we classify the footprint indicators into four patterns, the biologic, the atmospheric, the aquatic and the geologic. For example, the ecological footprint belongs to the first pattern, the carbon footprint to the second, the water footprint to the third, and the energy footprint to the last. For the chemical footprint, it depends on the exact pollutant pathways. A description of the boundary, function and characteristics for each pattern is actually is.

MOPC5-5
Established and emerging footprints – striving towards a valid and comprehensive support for decision-making processes
MOPC5-1
Development of a framework for assessing the chemical footprint
S. Sala, D.W. Pennengton
European Commision - Joint Research Centre, Ispra, Italy

The last few years, environmental footprint concept has obtained an increasing interest by both the scientific and political communities. The chemical footprint evaluation aims at assessing at which extent actual emission of chemicals harm the ecosystems above their capability to recover (the so-called carrying capacity of the system). According to the ecological footprint, but this time with a different focus, the chemical footprint is able to measure the environmental impacts from chemical processes and products, from different disciplines (from ecotoxicology to ecology, from ecological/human risk assessment to life cycle thinking and related tools), may support the future development of policy both for chemical protection and health economy. A review of existing approaches and methodologies able to be integrated in a chemical footprint framework will be presented and assessed. The following issues are the key elements: - Analysis and interpretation methods, Calculation algorithms and LCA tools, - LCA systems modeling, and inventories calculation, - Footprint components, - Assessment methods, - Calculation algorithms and LCA tools, - Analysis and interpretation methods, - Combination of LCA and other methods to assess products and services, - Surveys.

The co-founders are willing to see SCORE LCA growing thanks to new memberships from other companies. SCORE LCA is opened to any entity, and has the will to be useful to the practitioners and to be compliant with the need of the experts that will be disseminating the results of the research. SCORE LCA will drive collaborative research thanks to the annual funding of each member, and the potential complement of public funding.

The research topics will be defined and selected by SCORE LCA members, on an annual basis. An “Experts committee” will assist the members in this selection of topics. Each research project will be done by a selected organism (or group of organisms) such as research institutes or consulting company. They will be selected by SCORE LCA members through a call for tenders. Each project will be supervised by a specific panel formed by members and selected experts from the “Experts committee”. The set up of SCORE LCA will be done before end of 2011. The research topics that will be covered by SCORE LCA studies encompass the following points:

- Analysis and interpretation methods,
- Calculation algorithms and LCA tools,
- Indicators and impacts,
- Calculation algorithms and LCA tools,
- Analysis and interpretation methods,
- Combination of LCA and other methods to assess products and services,
- Surveys.

The interest to belong to SCORE LCA is to be able to participate in the definition of the research topics and to be able to monitor the studies. SCORE LCA is currently preparing call for tenders on two first topics. Contractors will be selected among the candidates at the beginning of 2012, and the work will start in 2013. A new call for tenders will be done in the future and expectations that researchers from all Europe will collaborate to fulfill these research projects.

The goal of the poster is to present the activities of SCORE LCA, including the two research topics that will be covered first. Some representative will be there to answer the questions from the attendees to the conference, including from the Universities or Research Laboratories which would like to be candidates to the first call for tenders.

MOPC5-2
The footprint family: differentiation, classification, and integration
F. Kai, R. Huygens
Leiden University, Leiden, Nederland

In the past decades, a variety of footprint concepts has entered the sustainability community under intensive debate. In this paper, we first review relevant literature. The idea of footprint, with the reference standard to each economic sector. This aspect plays a major role when applying different assessment methods, since results show important changes according to the set of resources and emissions included in the EE. However, the increase of scenarios with different EEIs does not substantially influence the results at the level of economic sector’s contribution. Whatever the scenario considered and the method applied, the financial and banking sector relieves to more than 45% to the consumption of Luxembourg. However, differences in the indirect contribution of the industrial sector are discussed with special emphasis on the benefits for potential applications in policy support.

MOPC5-3
Comparison of life-cycle-based methods to assess the consumption of Luxembourg
Public University of Luxembour, Luxembourg

A plethora of decision-making tools and sustainability indicators is available today for supporting national stakeholders and public authorities in the process of implementing resource management regulations and environmental impact mitigation strategies. The reliability of these methods, as well as the requirement for data collection and impact assessment methods is widely debated, especially for policy making support purposes. In this poster presentation, we intend to discuss several methodological approaches to identify a reliable framework to support reduction strategies (of emissions and resource use) at national level. More specifically, in this poster we present the assessment of a country’s net consumption (where Consumption=Production+Imports-Exports) using different life-cycle assessment (LCA)-based methods (i.e. Ecological Footprint, ReCiPe and Solar Energy Demand-SED) along with different environmentally extended input-output tables (EEIOTs) of EU-27, USA and The Netherlands.

The case study of Luxembourg is used to evaluate this methodological framework. By using EF, SED, and ReCiPe we cover the main environmental impacts generated by a country’s consumption. However, results highlight the need for higher consistency for different EFs. Indeed, there is a high uncertainty related to the type of environmental extensions (EE) coupled to each economic sector. This aspect plays a major role when applying different assessment methods, since results show important changes according to the set of resources and emissions included in the EF. However, the increase of scenarios with different EEIs does not substantially influence the results at the level of economic sector’s contribution. Whatever the scenario considered and the method applied, the financial and banking sector relieves to more than 45% to the consumption of Luxembourg. However, differences in the indirect contribution of the industrial sector are discussed with special emphasis on the benefits for potential applications in policy support.

MOPC5-4
Improving the relevance of carbon footprint calculations: temporal issues and global warming indicators
F. Kai, R. Huygens
Leiden University, Leiden, Nederland

In the past decades, a variety of footprint concepts has entered the sustainability community under intensive debate. In this paper, we first review relevant literature. The idea of footprint, with the reference standard to each economic sector. This aspect plays a major role when applying different assessment methods, since results show important changes according to the set of resources and emissions included in the EF. However, the increase of scenarios with different EEIs does not substantially influence the results at the level of economic sector’s contribution. Whatever the scenario considered and the method applied, the financial and banking sector relieves to more than 45% to the consumption of Luxembourg. However, differences in the indirect contribution of the industrial sector are discussed with special emphasis on the benefits for potential applications in policy support.

MOPC5-4
Specific data have been combined to obtain characterization factors for the resources indicator, developed in line with the substance flows of the ecoinvent database. Developments of new characterization factors for land occupation and transformation are based on the work of Koellner et al. (2008). The CML approach and country-specific life cycle impact assessment (LCA) approaches are used to determine the impact of land use on the environment.

Nine different indicators were selected to represent three different impact endpoints of land use: resource depletion, soil quality and biodiversity. The case study covered a beer production system with all the life-cycle stages from barley cultivation to the serving of beer at restaurant. Three optional system expansions were studied to estimate the warming potential of the entire system. For assessing land occupation and transformation LCI results, the ecological footprint and at least one of the biodiversity indicators are recommended. Regarding soil quality, the lack of reliable regional data currently limits the applicability of the methods proposed. The results show that the choice of a time horizon for the analysis or the use of an alternative metric can completely change the conclusions of a comparative carbon footprint study. Carbon footprint calculations often take place in an illustrative purpose, consumers and corporate decisions are often based on life cycle assessment (LCA) approaches that are developed to provide a framework for those calculations. There is a broad consensus to multiple greenhouse gas (GHG) emission by its GWP (Global Warming Potential) value, calculated for a chosen time horizon, and to add them up to get a carbon footprint expressed in kg CO2-eq. However, this approach implies some assumptions and simplifications that can bias the results and the conclusions drawn from them.

Recent papers have shown that the use of GWPs calculated for a given time horizon can lead to inconsistent analysis, and that the global warming impact can be very sensitive to different metrics and time-related issues. The use of a time horizon is a value-laden choice that can have significant impacts on the decisions taken from carbon footprint results. The increasing importance of some time-related phenomena, such as storing carbon in biomass or delaying GHG emissions, some researchers have shown that temporary carbon storage can have a beneficial impact on climate by decreasing cumulative radiative forcing over a given time horizon, but can cause an increase in temperature right after the end of the storage period.

In this study, a new approach is applied to test the hypothesis that the application of the CML approach is used to determine the time-dependent cumulative radiative forcing for each product. A new dynamic characterization factor is also developed to calculate the instantaneous increase in temperature caused by the life cycle emissions. This characterization factor is based on a spreadsheet model of Global Temperature trends and its results that were consistent with each other. An analytical expression to calculate the temperature increase at any time following a GHG pulse-emission. The results show that the choice of a time horizon for the analysis or the use of an alternative metric can completely change the conclusions of a comparative carbon footprint study. Carbon footprint calculations often take place in an illustrative purpose, consumers and corporate decisions are often based on life cycle assessment (LCA) approaches that are developed to provide a framework for those calculations. There is a broad consensus to multiple greenhouse gas (GHG) emission by its GWP (Global Warming Potential) value, calculated for a chosen time horizon, and to add them up to get a carbon footprint expressed in kg CO2-eq. However, this approach implies some assumptions and simplifications that can bias the results and the conclusions drawn from them.

The main advantage of this new approach is to propose a transparent method, easy adaptable in the future, and which covers impacts usually not included in LCA, such as indirect impacts, like those originated from related activities and services must also be addressed. For assessing the global impacts that this sector may have on the environment, the indicator Ecological Footprint (EF) can be used. The EF considers the energy and raw materials fluxes to and from any defined system or economy, transforming them into areas of land or water required for nature and generating of and/or absorbing these fluxes. One of the main advantages of EF is its ability to inform general public about the impact an activity has on the world’s biocapacity. EF analysis has been applied to nations, regions and to a wide variety of sectors and activities. Pressure of nations on marine ecosystems has also been assessed by this methodology.

A specific example of this methodology is the work of Galicia (SW Spain) is the largest fishing port of the world. Therefore, a representative part of the fishing extractive sector relies on the port activities. However, there are other important activities within the port which also requires resources consumption and thus, need to be evaluated. In this study, the environmental impact of the global activity of the Port of Vigo was assessed and a world-wide reference level was obtained which will provide information to the Port Authority on the main impact categories, in order to take the necessary measures to improve its environmental management strategy.

MOPC3-5

Ecofootprint of the Port of Vigo (NW Spain)
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Sustainability of the fisheries sector is nowadays a key issue, since current practices of this activity are depleting marine resources and endangering biodiversity. Besides, indirect impacts, like those originated from related activities and services must also be addressed. For assessing the global impacts that this sector may have on the environment, the indicator Ecological Footprint (EF) can be used. The EF considers the energy and raw materials fluxes to and from any defined system or economy, transforming them into areas of land or water required for nature and generating of and/or absorbing these fluxes. One of the main advantages of EF is its ability to inform general public about the impact an activity has on the world’s biocapacity. EF analysis has been applied to nations, regions and to a wide variety of sectors and activities. Pressure of nations on marine ecosystems has also been assessed by this methodology.

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MOPC3-6

Ore grade decrease as an indicator to address mineral resource scarcity in life cycle assessment
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Resources are a topic of growing importance in political agenda. Mineral resources have dissipative use meaning that they are available on Earth but often degraded or dispersed. This way, mineral resources extraction will invariably continue and so will the decline of ore grades for all minerals worldwide, given no new discoveries (Mudd, 2009). Consequently, in order to extract the same amount of mineral, higher volumes will be required. Ore grade is an inherent property of mineral resources and yet it also expresses the scarcity of the resource. Life Cycle Assessment for ore grade decrease has not been used as an indicator directly in the literature up to now. The goal of this paper is to develop a meaningful life cycle impact assessment method to assess mineral resource scarcity and to calculate characterization factors for ten minerals.

The method here proposed expresses the decrease in ore grade as a response to an increase in production. Characterization factors for mineral resource use were defined as the marginal decrease of ore grade per unit of mineral extracted (as response to the additional amount extracted of this mineral). We calculated for the following ten element-group's characterization factors: cobalt (Co), copper (Cu), diamond (D), gold (Au), iron ore (Fe), lead (Pb), molybdenum, (Mo), nickel (Ni), silver (Ag), zinc (Zn) and platinum-group metals. Production volumes and ore grade data per mine between 1998 and 2010 were retrieved from the Raw Materials Database developed by the Raw Materials Group. Results for ore grade are presented in fraction of ore grade decrease per mass of mineral extracted. A life cycle study will be included to illustrate the applicability of the method proposed.

MOPC3-7

Applicability of land use impact assessment methods in LCA - findings from practitioners' perspective
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Inclusion of land-use related environmental aspects in LCA has been under active development in recent years. Many indicators have been developed and proposed for different aspects of land use but have, as yet, not been tested and compared in LCA applications. Aim of this study was to test the applicability of the general framework and several selected land-use LCIA indicators in an illustrative case study from practitioners’ perspective.

Nine different indicators were selected to represent three different impact endpoints of land use: resource depletion, soil quality and biodiversity. The case study covered a beer production system with all the life-cycle stages from barley cultivation to the serving of beer at restaurant. Three optional system expansions were studied to estimate the possible impacts of substituting feed protein (soybean, rapeseed and silage) with mash co-product from brewing. A comparison with wine production was also made for illustrative purposes.

All tested land-use indicators were applicable in LCA. Some indicators were found to be highly sensitive to assumptions on land transformation, which sets high requirements for data. A life cycle impact assessment method set up for the case study is presented.

MOPC3-8

Development of biodiversity and resources indicators assessing potential impacts throughout the life cycle of products, processes and services
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While carbon and water footprint indicators begin to be fully integrated in the decision making process of industrial stakeholders, the question is still effective for other potential environmental impacts such as biodiversity and resources. Within Life Cycle Assessment (LCA), existing impact assessment methods do not fully address this issue when communicating to non-LCA practitioners, because of complex units and only partly integrated issues (e.g. arable land as a resource, not integrated in impact assessment methods so far).

The objective of this study is to propose a methodological framework which has been established to take into account the environmental performances of products, processes or services using two indicators: resources and biodiversity. The biodiversity indicator contains aquatic ecoxotoxicity (USEtox), terrestrial ecoxotoxicity, aquatic eutrophication, aquatic acidification, terrestrial acidification/nitrification (IMPACT 2002+), impacts of turbined water, cooling water, and a new framework for land occupation and transformation. The resources indicator includes the impact on the stock of the extraction of minerals, fossil fuels, peat, and overexploitation of wood and arable land.

Developments of new characterization factors for land occupation and transformation are based on the work of Koellner et al. (2008). The CML approach and country-specific data have been combined to obtain characterization factors for the resources indicator, developed in line with the substance flows of the ecoinvent database. The potential impacts of the 4000 ecoinvent processes have been calculated and statistically analysed to point out any inadequacy in the methodology regarding the operational data quality. Challenges have been faced in obtaining reliable data in the inventory and impact assessment phase. The majority of the land-use impacts occurred in the cultivation phase, but significant impacts were also found down the supply chain. Scarcity of land-use LCI data sources limits validation and cross-comparison. Interpretation of indicator results is complicated due to limited understanding of the environmental impact pathways of land use. Most of the land-use indicators led to results which were not consistent with each other.

None of the tested indicators describes the full range of environmental impacts caused by land use, nor fulfills all the criteria for an effective ecological indicator. Many well-known general LCA method sets include an impact category 'land use', but fail to cover all three impact endpoints. We recommend presenting land occupation and transformation LCI results, the ecological footprint and at least one of the biodiversity indicators. Regarding soil quality, the lack of reliable regional data currently limits the application of the proposed methods. The criteria for effective ecological indicators should be reflected in further work in indicator development. Development of regionalized characterization factors is of key importance to include land use in LCA.
A set on pollinators: feedback and basis for the future

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Pest Management Regulatory Agency, Ottawa, Canada
US Environmental Protection Agency, Washington, United States of America
Bayer CropScience, Washington, United States of America
Syngenta Ltd, Bracknell, United Kingdom
Pennsylvania University, Pennsylvania, United States of America
Australian Environmental Agency, Barton, Australia
ESA, Paris, Italy
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Swiss Bee Research Center, Switzerland
Xeres Society for Invertebrate Conservation, Portland, United States of America
BASE, US, United States of America

There have been reports, in recent years, of declines in native and managed pollinators in several regions of the world. Modern crop management practices, progressively implemented in order to allow the extension of cropped areas and ensure a proper control of pest populations and diseases have often been pointed as a factor responsible of this decline. Plant Protection Products (pesticides) are part of these management practices. Thus regulatory texts have been adopted all around the world, requiring an assessment of the impact of these products on the agricultural environment, and among others on arthropod and pollinating species.

Concern regarding the extent to which the use of pesticides in crop protection affects pollinators varies among countries. The perception we have on pollinating species appears to result from an initial knowledge on their implication in crop protection and related consequences of a temporary decline on the crops of concern. With regard to the three subgroups, we refer to the non-target terrestrial invertebrates, to the non-target aquatic invertebrates and to the non-target aerial invertebrates (i.e. fish, aquatic invertebrates, algal, aquatic plants, birds etc). A global Pellston SETAC workshop on estimating the potential risks of plant protection products to insects was organized in January 2011, to bring together the best available science regarding exposure and effects assessment methodologies for Aps and non-Aps species, and harmonize further the risk assessment approaches among North and South America, Europe, Asia and Africa for a global improvement of the protection of insect pollinators in cropped area.

The proceedings of this workshop will be finalized in the coming weeks and this presentation aims at offering the audience an outline of these proceedings.

Agricultural pesticides (plant protection products) are part of these management practices. Thus regulatory texts have been adopted all around the world, requiring an assessment of the impact of these products on the agricultural environment, and among others on arthropod and pollinating species. As a complement to this assessment, appropriate feedback from the field as an indicator of possible side-effects of pesticides on pollinating species and of conditions of pesticide use that ensure limited exposure is essential to evaluate the effectiveness of risk management measures. Finally, early alerts and clear communication of key research findings related to pollinators are also needed to allow fast and effective decisions.

In this context, the creation of a dedicated working group was approved by the OECD Working Group on Pesticides (WGIP) in May 2010. The objective given to this "Pesticides Effects on Insect Pollinators" (PEIP) Expert Group is to develop a toolbox for risk assessment, risk management, incident reporting, and research about pollinators, focusing on honey bees and non-Aps bees and on the specific case of pesticides as potential factor of stress.

Activities of the OECD expert group on pesticide effects on insect pollinators in the context of the risk assessment for pesticides
A.O. Alix 1, A.P. Pease 1, F.P. Delorme 2, J.H. Hart 3, S.F. Poret 4

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There have been reports, in recent years, of declines in native and managed pollinators in several regions of the world. Modern crop management practices, progressively implemented in order to allow the extension of cropped areas and ensure a proper control of pest populations and diseases have often been pointed as a factor responsible of this decline. Agricultural pesticides (plant protection products) are part of these management practices. Thus regulatory texts have been adopted all around the world, requiring an assessment of the impact of these products on the agricultural environment, and among others on arthropod and pollinating species. As a complement to this assessment, appropriate feedback from the field as an indicator of possible side-effects of pesticides on pollinating species and of conditions of pesticide use that ensure limited exposure is essential to evaluate the effectiveness of risk management measures. Finally, early alerts and clear communication of key research findings related to pollinators are also needed to allow fast and effective decisions.

The OECD Food and Environment Agency (OEFA) is responsible for the science behind the development of a risk assessment guidance of plant protection products on bees (Aps mellifera, Bombus spp. and solitary bees). F. Streissl 1, R.L. Lurte 2, C.S. Stent 2, D. Auteri 4

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A decline in pollinators was reported from several different regions of the world. Pollination is a very important ecosystem service for food production and maintenance of biodiversity. Pesticides are one of the factors that may contribute to the decline of pollinators observed worldwide. This has led to concerns that the current risk assessment for pesticides need revision. As a result, this regulatory challenge the European Commission tasked EFSA to develop an updated Guidance for pesticide risk assessment and bees. The opinion on the science behind the risk assessment for bees analyzes the existing test protocols and risk assessment schemes. It gives recommendations on how to assess cumulative and synergistic effects and how to assess the risk of substances which are highly toxic to bees such as neonicotinoids. The approach of definition of specific protection goals is used the first time in the context of developing pesticide risk assessment. The present opinion of the PPR panel provides the scientific basis for the final Guidance Document of EFSA.

Activities of ICPBR in the context of the risk assessment for pesticides to bees

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The International Commission for Plant- Bee Relationships (ICP-BR) organizes working sessions in Europe on the harmonization of methods for testing the toxicity of pesticides to bees for over 30 years. ICP-BR develops the scientific process underlying decisions from European administrative authorities, EFPO (European and Mediterranean Plant Protection Organization) and the European Commission for Economic Cooperation and Development. ICP-BR has acquired considerable authority in the area of legislation and regulation concerning beehive protection related to the use of plant protection products, bringing together the European expertise of national authorities, industry, research and beekeepers. The Bee Protection Group held its first meeting in Wageningen in 1980 and over the subsequent 30 years evolved into a well established forum for addressing the risk of pesticides to bees. It has operated by reaching consensus amongst a wide range of experts active in this field drawn from industry, regulatory authorities and research institutes across the European Union (EU). Operating through the EFPO honey bee sub-group, it has produced the testing methodology and risk assessment guidance currently used under Directive 91/414/EEC and Regulation 1107/2009/EC. Returning to Wageningen in November 2011, this re-established expert group at the Honeybees event where research in the area of pesticide effects on bees was presented and where implications for risk assessment were discussed and taken on board in working group activities. This presentation aims at giving an overview of ICP-BR activities with an emphasis on each of the seven working groups (testing effects on bee brood; semi-field and field testing; acceptability of effects in testing; statistics; risks related to sowing dusts; risks of guttation and monitoring (studies) activities).

Activities of the OECD expert group on pesticide effects on insect pollinators in the context of the risk assessment for pesticides

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Review of higher tier methods for assessment of the risk of pesticides to honey bees
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Risk assessment have been developed for domesticated honeybees (Aps mellifera) potentially exposed to residues of insecticides. After initial tier I screening risk assessment further studies may be conducting for those substances for which a potential risk cannot be excluded. Higher tier studies are often performed in cages or tunnels containing colonies of honey bees which can forage on treated crops. Cage test are typically smaller in size than tunnels but allow for the testing of more treatments often with more replicates than the smaller scale tunnel under more realistic conditions but due to use of free flying colonies there is less control than in the tunnel and cage studies. Finally, post-registration monitoring can offer additional information on specified uses of the product under commercial conditions and can be used to feedback on the outcome of the risk assessment and the risk management practices put in place to protect bee. This paper will review the various higher tier methods with respect to their ability to accurately produce the potential effects of pesticides to honey bees and how they can be employed in a robust risk assessment. The range of parameters investigated typically include; mortality, foraging, behavior, brood and colony.
Assessing the comparative risk of plant protection products to honey bees, non-target arthropods and non-apis bees
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Risk assessments are conducted for plant protection products (PPP) with respect to potential impacts on non-target species. These include pollinators such as the honey bee but also other non-target arthropods (NTA). In common with other areas of ecotoxicological risk assessment sentinel species are employed aiming at ensuring a high level of protection. Tier I screening risk assessments are intended to rank and exclude those substances which pose a low risk to non-target species and to focus resources on those for which a potential risk cannot be excluded and further studies may be undertaken to characterize the conditions and occurrence of risks. In the European Union a Hazard Quotient (HQ) approach is used to assess the risk to both honey bees non-target arthropods at Tier I. This is calculated by dividing application rate by the toxicological NOEC (No Observed Effect Concentration). In other regions such as North America, a contact toxicity trigger of 11 µg active substance/bee is currently employed. At the Pelliston workshop it was suggested that for a risk assessment for sprayed products the honey bee could be a suitable surrogate species. However, to account for potential differences in the sensitivity of the honey bee as a test organism and other non-Apis bees a safety factor of 10 (for interspecies differences) was suggested. On the basis of the effects of toxicological data of the PPP on bee and NTA, the relative risk of PPP to NTA, honey bees and non-Apis bees, as depicted by HQ values is compared and where possible data on NTA pollinators (e.g. Syrphids) and non-Apis bees (e.g. Bombus sp.) are also included. At Tier I using HQ trigger of 50 for honey bees, 20 for NTA, in accordance with the current EU legislation, and 5 (i.e. 50 divided by the extra factor of 10 abovementioned) for non-Apis bees, the NTA scheme identifies substances to move to Tier II for further evaluation. The suggest non-Apis HQ trigger to give a similar pass / fail rate to the NTA scheme but was slightly confounded by a lack of determined end points. However, even this taking into consideration the most restrictive tier I assessment was that for NTA. This poster examines the potential for a Tier I risk assessment to cover NTA, honey bees, non-Apis bees and other arthropod pollinators as part of a tiered risk assessment scheme and compare the empirical HQ approach with methods using more specific species exposure estimates.
Effects of propylparaben during medaka (Oryzias latipes) embryo development using in vivo morphometrical/quantitative approaches
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Parabens are antiemulsifiers preservatives widely used in personal care and food products. Among them, the most used are generally methyl and propylparaben (PB) which in turn, turns to be the most detected in aquatic ecosystems. Besides the numerous reports about the in vitro and in vivo estrogenic activity of PB and other parabens, relatively few studies have been conducted to determine sublethal effects to aquatic organisms. In this sense, the use of fish embryos and eleutherembryos is promising alternatives to existing ecotoxicity tests without reducing the validity of the risk assessment. These stages also provide the application of in vivo methods for the analysis and quantification of biomarkers (DNA adducts, estrogen receptors). In this paper we propose a battery of non-invasive morphometrical tools to examine physiological and anatomical features in embryos and eleutherembryos of medaka (Oryzias latipes) so the effects of waterborne exposure to PB (40-400 µg/L) can be investigated. Preliminary findings show PB ≥ 400 µg/L resulting in significant quantitative differences, although these exposure effects may not necessarily compromise fish normal development. Further studies will be needed to assess whether these effects may result in long term toxicity. This work is being made possible thanks to Spanish Government Grants RTA2010-00004-C02 and CTS2010-17779-C02
TH 006
Evaluation of genotoxicity of sediment samples using zebrafish embryo and comet assay
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Genotoxic hazard estimation of sediment-bound anthropogenic pollutants is still a challenge since many genotoxics tend to adsorb onto particulate matter. Testing of extracellular genotoxic risk because bioavailability of the compounds is not considered and enrichment processes like Solshei extraction might lead to false positive results due to artifact formation. For genotoxic risk assessment often biomonitoring experiments with in the field caught fish are performed. The in vitro zebrafish embryo model represents an alternative scenario since it offers the possibility to simulate in situ exposure scenarios and is considered as a replacement to animal experiments according to current legislation. In this work the novel method for determination of genotoxicity induced by particle-bound pollutants is presented. Therefore zebrafish embryos were exposed to whole sediments and DNA damage was assessed by the comet assay in the whole embryo test in whole body cells (Kosmol6). Preliminary results showed a high intra-assay variability of DNA damage since a heterogeneous cell mixture from homogenized tissues was obtained. Nevertheless, the in vitro fathead minnow model turned out to be a sensitive tool for detecting total genotoxic hazardous potential of sediments. Even though it should be considered that the egg chorion represents a selective barrier for toxic compounds. As well, the metabolic competence of the embryos, which is closely related to the stage of development, demands further investigations by varying exposure windows.
TH 007
Modeling concentration-response relationships of quantitative gene expression in zebrafish embryo and its application to predict mixture effects
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Chemicals in the environment are rarely found as single compounds but rather as part of (complex) mixtures. Mixtures may provoke effects which are difficult to describe based on the knowledge of effects for single compounds. Over the last decades several models (concentration addition, independent action) have been tested and applied to predict mixture effects using apical endpoints like lethality or growth inhibition. However, the effects of mixtures at a molecular level are still poorly understood. Using zebrafish embryos we aim to investigate response patterns in zebrafish embryos Initialy we analyzed effects of single substances on the gene expression of specific genes to model concentration-response relationships. Based on these results we will conduct mixture experiments investigating whether or not existing models for apical endpoints can be applied for molecular responses as well. Finally the modeling approach will be extended to the entire transcriptome level by analyzing the effect of mixtures using microarrays. The overall goal is to apply the findings for a better interpretation of gene expression patterns provoked by exposure to environmental samples.
First results clearly show that gene expression follows mononitrogen concentration-response relationships. However, at higher concentrations (above LC50) we noticed an inhibition on the test concentration-variable responses. We compared classical toxicological models such as the sigmoidal Hill-slope to peak and other alternative models. To date we have completed the detailed analyses of 4 different chemicals that have been identified by a previous microarray analysis as sensitive and specific markers for exposure to chemicals with different modes of action.
TH 008
An evaluation of the zebrafish embryo model for estrogenicity testing of wastewater treatment plant effluents
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Wastewater treatment plants are known sources of water pollution which may cause endocrine disruption in fish and other aquatic biota. Wastewater treatment plant effluents contain complex mixtures of compounds with various estrogens, xenoestrogens, antiandrogens, and other compounds which may potentiate each other. Therefore to model overall potential estrogenic effects in fish, it is necessary to test the whole mixture (rather than assessing individual chemicals alone). To measure estrogenic effects in the effluents, we have combined fathead minnow embryo with quantitative RT-PCR. Modulations of three estrogen responsive genes have been evaluated including specifically aromatase B, vitellogenin 1, and estrogen receptor α. The effects have been tested with series of waste water treatment plant effluent samples collected during the Swedish monitoring campaign (coordinated by the JRC, Ispra, Italy), and the results obtained with the fathead minnow bioassay have been compared to comprehensive analytical data of various contaminants. The present work contributes to the characterization of the zebrafish model as a tool for investigation of in vivo estrogenicity in complex contaminated samples. [The work has been supported by Brno PhD Talent Financial Aid to Adam Jonáš and the projects INCHEMBIOL (MSM0021622412) and CETOCOEN (CZ.1.05/2.1.00/01.0001)].
TH 009
The normal development of the fathead minnow
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The fathead minnow (Pimephales promelas), the zebrafish (Danio rerio) and the Japanese medaka (Oryzias latipes) range among the most commonly used fish species in aquatic toxicology. However, acute tests (OECD test 203) require the use of large numbers of test animals. In order to reduce the number of test animals, alternatives are under development to establish the embryo zebrafish test (ZFET) as an alternative to conventional acute fish toxicity testing. Whereas the ZFET is currently undergoing OECD validation, fathead minnow embryos have only scarcely been used for embryo toxicity testing. Based on fathead minnow staging was performed in petri dishes at 25±1°C. Pictures were taken every 5 minutes between 0 - 1 hpf and every 30 minutes from 3 to 12 hpf. Pictures of embryos older than 12 hpf were taken every 60 minutes. After initiation of spontaneous movements (approx. 26 hours post-fertilization (hpf)), the embryos were dechorionated using 2 mg/ml pronase solution and anaesthetized with 0.016 % tricaine solution. In fact, it turned out that most of experimental parameters could be mimicked well by the ZFET. Furthermore, we have in our database generated concentration-response data (fathead minnow embryos) to a battery of non-steroidal and weakly estrogenic compounds like dichloroaniline, which is currently used as the positive control in the ZFET and a substituted phenol, (2,4-dichlorophenol). Fathead minnow embryos proved to be slightly less sensitive for cadmium chloride and definitely less sensitive for 2,4-DCP than adult fathead minnow (TG 203), whereas for 2,4-DCP no difference in sensitivity could be detected. However, the use of the ZFET as a rapid and non-invasive test to investigate the effects of waterborne exposure to PB (40-400 µg/L) can be investigated. Preliminary findings show PB ≥ 400 µg/L resulting in significant quantitative differences, although these exposure effects may not necessarily compromise fish normal development. Further studies will be needed to assess whether these effects may result in long term toxicity. This work is being made possible thanks to Spanish Government Grants RTA2010-00004-C02 and CTS2010-17779-C02
TH 010
A modified approach for the determination of biocorcentration factors (BCF) in fish
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The method for the determination of biocorcentration factors (BCF) given in OECD guideline 305 builds on the exposure of adult fish to a dilute aqueous solution of chemicals at a constant concentration. Thus, numerous samples of water and fish tissue have to be analyzed to calculate the BCF. The number of fish killed during the test is high (average 30 and is expensive. Due to the high number of fish used for BCF determination, many alternative methods have been developed with the aim to facilitate the screening for BCF, e.g. with fish larvae or with fish eggs and 14C-labeled compounds. In the ProDart project funded by the German Federal Ministry of Education and Research (BMBF, project no. 315399), the Helmholtz Centre for Environmental Research (UFZ) and the laboratories of the Federal Environment Agency (UBA) made a further attempt to determine BCF and to further reduce both effort and the total amount of test animals. Moreover, a method of sample preparation prior to gas chromatography-mass spectrometry analysis (GC-MS) was developed. So far the approach has been tested
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with phenanthrene, chlorpyrifos and methyl triclosan.

Aqueous stock solutions of the chemicals were prepared in pure water. An aliquot of each stock solution was diluted in the dilute solutions at 28°C. At the start of exposure and also 1, 24, 30, and 48 hours later the fish were sampled for lethal and sublethal toxicological endpoints. Furthermore, samples of the aqueous medium were taken. The extracts were compounded by solid phase extraction (SPE), and analyzed with GC-MS. Samples of fish eggs were taken at 24 and 48 hours after the start of exposure. The eggs were smashed in ethanol and treated in an ultrasonic bath. These extracts were concentrated and analyzed with GC-MS. Concentrations in the aqueous medium were compared with the residues detected in fish eggs and degradation curves were calculated.

TH 01.01
Exploring animal alternatives: seeking a replacement for whole effluent toxicity testing in fish

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In test facilities discharging effluents directly to surface waters must conduct periodic evaluations of whole effluent toxicity (WET). One method commonly used to obtain WET data is the 7 day larval growth and survival test (LGS) in which newly hatched fathead minnows (FHM, Pimephales promelas) are exposed to effluents for 7 days with mortality as an endpoint. Given the importance of animal welfare in toxicity testing, the current study aimed to identify a test method that is able to fulfill the requirements for WET data, while minimizing the use of protected organisms or life stages. One possible alternative is the fish embryo toxicity test (FET). The FET has already been sanctioned for WET testing in the European Union (EU) using embryonic zebrafish (Z. freni, Dario retio); however, the FET to be adopted in the United States, it must be applicable to species commonly used in contract laboratories (i.e., FHM) and demonstrate comparable sensitivity to the LGS. Thus, the main objective of this study was to develop alternatives for the FET and the LGS, to harmonize the FET-related endpoints (FHM-LGS and 2) and to compare the sensitivity of the FET and the LGS using both FHM and ZF. To accomplish the first objective, tests using existing protocols for the FET and LGS were carried out with both species under different temperature and feeding regimes in order to determine optimal conditions for testing. The results were then validated and compared to the results of the LGS in order to provide an alternative to the FET for testing WET in the US. The adoption of FET testing for this purpose would result in the use of an earlier life stage to obtain sub-chronic toxicity data, representing a refinement of current test methods in the spirit of the three R’s.

TH 01.02
Final results of the OECD transferability study on the transdisciplinary, intra- and inter-laboratory reproducibility of the Zebrafish Embryo Toxicity Test

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The OECD acute fish toxicity test guideline (TG 203) is an integral component in the environmental safety assessment of industrial chemicals, agrochemicals, pharmaceuticals and biocides. One of the most promising approaches to the acute fish toxicity test is based on the use of zebrafish embryos. In 2005, the former German Environmental Agency submitted the draft TG on ‘Fish embryo toxicity test (FET)’ to the OECD test guideline program and a supportive draft detailed review report. Subsequently, the OECD established the ad hoc Expert Group on the Fish embryo Toxicity Test. Based on the outcome of expert meetings, OECD decided to perform validation studies by European laboratories. The validation studies aimed at evaluating the transferability, and the intra- and inter-laboratory reproducibility of the Zebrafish FET (ZFET). Newly sensitised zebrafish embryos (20/concentration and control) were exposed for up to 96 h to chemicals. Four apical endpoints are recorded daily as indicators of acute lethality in fish: coagulation of the egg, lack of formation, non-detachment of the tail bud from the yolk sac and lack of eye pigmentation. The values are calculated for 48h and 96h exposure. During the validation, 20 chemicals were tested at 7 different concentrations in 3 independent runs at least 3 laboratories with appropriate controls. Stock solutions and test concentrations were analytically confirmed for 11 chemicals. The poster will give an overview on the validation study design, the results and the correlation of the ZFET with acute fish LC50 data. Acknowledgments to the participating scientists (laboratories): Edward Salinas (BASF, Germany), Fernando Martinez-Jeronimo (Instituto Politecnico, Mexico), Przemyslaw Fochman & Helena Rzodeczko (Ipo-Pszczyna, Poland), Juliette Legler & Peter Cenijn (IVM, The Netherlands), Nicole Haebl & Stefan Weigt (Merck Serono, Germany), Leo van der Ven & Evert Jan van den Brandhof (RIVM, The Netherlands), Carolia Kussatz & Christian Polleichten (UBA, Germany), Stefan Scholz (UFZ, Germany), Hilda Wittmers (UET, Belgium). "Disclaimer: The opinions expressed and the arguments employed herein are those of the authors and do not necessarily reflect the official views of the OECD or the governments of its member countries, or the European Commission!"

TH 01.03
Genotoxic effects of sediment extracts and native samples from Hamburg harbor and the Rhine River in Danio retio embryos

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The European Water Framework Directive (EWFD) aims to achieve a good ecological and chemical status in the surface water of European rivers. However, since particulate matters and sediments are highly relevant as secondary sources for environmental pollutants, applied sediment toxicology plays an important role in ensuring the successful implementation of the goals of the EWFD. The present study is part of the joint research project DanTox, which - among other specific endpoints - investigates genotoxic effects of sediment extracts in zebrafish (Danio retio) embryos. The comet assay is a widely used bioassay gaining increasing popularity as a screening test for genotoxicity. However, the biological significance of the comet assay results in not yet clearly further correlations with biotests with definite endpoints such as the micronucleus assay are recommended. Within this study both tests will be compared, in order to gain a reliable genotoxic assessment of environmental samples.

Sediment samples were taken from the Rhine River (Ehrenbreitstein and Altrip) and Hamburg harbor (outer Vering Canal). After Soxhlet extraction of freeze-dried sediments, aqueous stock solutions for samples were prepared. The concentrations of chemicals in the aqueous medium were compared with the residues detected in an ultrasonic bath. These extracts were concentrated and analyzed with GC-MS. Concentrations in the aqueous medium were compared with concentrations in the filtrates and sediments of standard fish tests exposed to the same chemicals. Concentrations in the aqueous medium were compared with concentrations in the filtrates and sediments of standard fish tests exposed to the same chemicals. Concentrations in the aqueous medium were compared with concentrations in the filtrates and sediments of standard fish tests exposed to the same chemicals.

TH 01.04
The threshold approach for acute fish toxicity testing of formulated plant protection products: a proposed framework

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The EU has recently published guidance on the use of the threshold approach for fish acute toxicity testing. Essentially the approach uses a limit test at a single threshold concentration determined by the results of Daphnia and algae tests. If no mortality is observed in the limit test the fish acute value can be expressed as greater than the threshold value. However, mortality observed in the full concentration-response test is triggered. To assess applicability to plant protection formulated products, Syngenta conducted a database of 185 products (fish, Daphnia and algae endpoints) and retrospectively applied the threshold approach. This representative dataset demonstrates that a reduction of approximately 38% of the fish might be achieved. Furthermore, the approach also provides a refinement as in 76% of threshold tests no mortality would be observed and in 64% of cases there would be no toxic effects at all, including sub-lethal symptoms. However, there is a need for regulatory acceptance of this method particularly in relation to the use of the data in risk assessment, where a fish specific assessment is required and approaches may require a better defined estimate of chemical toxicology. Consequently, the UK NCIRs held a workshop in December 2010, bringing together industry and a number of European regulators to discuss the applicability of the approach. The workshop discussed a potential framework for applying a threshold type approach, incorporating all the available information including relative fish sensitivity, data from the work presented, ecotoxicological endpoints, formulated product information (Daphnia and algae) and potentially the Predicted Environmental Concentrations. Such an approach may be used to better define a fish threshold approach that is workable within the EU regulatory framework for Plant Protection Products.

TH 01.05
Different test conditions for hydrophobic and fugitive substances in the Fish Embryo Toxicity Test for a reliable risk assessment

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Background: Toxicity of chemicals is part of the DanTox project, which aims to identify specific toxicity and molecular modes of action of sediment-bound pollutants and selected primary pollutants using zebrafish embryos. Ecotoxicological investigations of volatile and hydrophobic chemicals in tests with aquatic organisms are difficult to conduct, since they evaporate from the water and/or adsorb to plastic surfaces. This could lead to substance loss during exposure, which can cause an underestimation of their short-term and hazardous potential. In order to optimize test conditions for such chemicals, the aim of this study was to compare embroytoxic effects caused by selected chemicals under static and semi-static test conditions in glass and plastic vessels.
Material and methods: The embryotoxicity of three selected substances (benzofuran, quinoline and chlorpyrifos) were tested in plain and glass vessels under static and semi-static conditions using the fish embryo toxicity test with zebrafish (Danio rerio). For semi-static tests, the solutions were exchanged every 24 h. Embryotoxicity were observed after 24, 48, 72 and 96 h post fertilization (hpf).

Results: Mortality at 96 h in plain vessels reached a significant higher mortality under static than under semi-static conditions after 72 hpf. In contrast, tests in glass vessels showed a significant higher mortality under static than under semi-static conditions, which was not expected. Quinoline showed in glass vessels a slightly higher toxicity under static than under semi-static conditions and a significant higher mortality under static than under semi-static conditions in tests conducted in plastic vessels. In contrast to benzoquinone, quinoline showed minor differences in toxicity with increasing exposure time. Differences in toxicity between quinoline and benzoquinone are probably based to different vapour pressure as well as Henry’s law constant. Chlorpyrifos showed only a low mortality (< 30 %) under all test conditions.

Conclusion: Only some significant differences, but mostly just tendencies between the different test conditions were recorded. Thus, no precise recommendation can be given for the selection of the most suitable volatile and adsorbable heterocycles. However, for further investigations chemical analyses are required to determine the difference between nominal and measured concentrations under all test conditions.

Acknowledgement - the authors thank the Federal Ministry of Education and Research for financing this project.

TH 016
A quantitative and mechanism specific toxicity assessment for the fish embryo test, using fluorescent zebrafish

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The fish embryo test (FET) is currently the most advanced animal alternative test in ecotoxicology. To date, the application of the zebrafish embryo toxicity test (FET) has focused on acute toxicity assessment, where only lethal morphological effects are accounted for. An application of the FET beyond acute toxicity, however, necessitates the establishment of more refined and quantifiable toxicological endpoints. A valuable tool in this context is the use of gene expression-dependent fluorescent markers and specific biological structures, which can help elucidating the underlying toxicological mechanisms and enhance the detection of adverse effects and the sensitivity and specificity of the conventional FET.

This project investigates the benefits of using fluorescent marker applications on zebrafish to study chemical toxicity on vascular, myotomal and neuronal development, thereby improving the FET.

The transgenic line Tg(fli1:EGFP)y1 expresses enhanced GFP in the entire vasculature under the control of the fli1 promoter, and thus enables the visualisation of vascular defects in live zebrafish embryos. We assess the fli1 driven EGFP-expression qualitatively and quantitatively, and found an exposure concentration related increase in vascular defects in zebrafish embryos and larvae. The effects are now being confirmed by QPCR. We additionally explored whole-mount immunofluorescence to visualise myotomal defects, using a monoclonal antibody staining specifically myosin in the embryonic muscle fibres. These investigations showed a chemical dependent change in muscle morphology for fli1 and genistein, what also could be quantified and declared as a robust parameter. Presently, another transgenic zebrafish line is being evaluated, expressing GFP coupled to the glial fibrillary acidic protein (GFAP) gene. The Tg(fli1:EGFP) embryos promise the identification of neurotoxic chemicals in the zebrafish model, and data will be presented.

The use of fluorescent transgenic lines and antibody staining enables the detection of chemically induced vascular, myotomal and neuronal malformations in the embryos, which were otherwise not visible in bright field assessment. The fluorescent signal based methods allow a sensitive and quantitative effect assessment, which will broaden the scope and minimize the data variability of the FET.

TH 017
Toxicity of chlorine-produced oxidants to Arabian killifish embryos for acute developmental stage exposures

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Fish are used as bioindicators for studies aimed at the understanding of toxic mechanisms and the indication of possible acute and chronic effects. Therefore, we present the use of Arabian killifish (Aphanius dispar) embryos, an indigenous species to the Arabian Gulf, to study the effect of chlorine-produced oxidants to marine organisms in the Qatari Coastal Area. The objective of this study is to develop chlorine toxicity data for the marine Fish Embryo Toxicity (mFET) test. The test is designed as a means to replace the fish embryo test (FET) for aquatic and terrestrial risk assessments. The embryonic and larval stages of killifish are used to study the acute toxicity to chlorine in semi-static conditions using the fish embryo toxicity test (FET) for up to 96h. The investigated endpoints included: coagulated eggs, somite development, heartbeat, tail detachment, hatchability and post hatch mortality.

The results showed a developmental stage-dependent response to chlorine. During earlier developmental stages, chlorine had reduced effects on the embryos and the survival rate and hatchability were high, even at relatively high concentrations. In later developmental stages (pre to post hatch, eluteroembryo), the embryos were significantly more sensitive to chlorine than in the early stages of development. The chorion, (membrane surrounding the egg), is believed to provide a barrier against chlorine in early stages of embryo development. Taking into account the findings presented here, Killifish embryos exhibit the ability to be an indicator organism for environmental risk assessments of the Qatari Coastal Area.

Benefits include, animal alternative, ease of fish breeding, clarity of the embryos, reduced sample size, reduced waste generation and shorter study duration.

TH 018
Sub lethal effects of methyltestosterone in adult zebrafish (Danio rerio)


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Methyltestosterone (MT) is a synthetic hormone used in fish-farming to induce male monosex in Oreochromis niloticus. In Brazil, the commercial hormone is illegally used as a sex change agent. Therefore, an approach for monitoring MT in the aquatic environment is needed.

In this study, we aim to evaluate the sub lethal toxicity of commercial MT (cMT) and its active ingredient 17α-methyltestosterone (aMT) to adult zebrafish. Comparison between the two compounds (cMT and aMT) is relevant for the regulation of MT in Brazilian aquaculture. For this purpose, sub lethal and tolerant concentrations were chosen as genotoxicity markers and measured on peripheral erythrocytes cells of zebrafish and cholinesterase-CHCh, lactate dehydrogenase-LDH and glutathione-S-transferase-GST were chosen as biochemical markers and measured in different fish organs.

Fish were exposed during 4 days at concentrations between 4 and 5000 µg/L of cMT or aMT.

Results showed that the most responsive biomarker tested was GST with a dose-dependent inhibition verified in several organs. Fish exposed to cMT during 4 days at concentrations between 4 and 5000 µg/L of cMT or aMT. Due to the increase of salinity derived from sea level rise, which may be occurring in aquatic organisms affected by MT effluents. Finally, the apparent higher effectiveness of cMT should be carefully assessed so that proper legislation can be established for MT use in aquacultures.

TH 019
Comparative sensitivity of different early life stages of zebrafish to copper and NaCl

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Aquatic contamination is a major concern to the health of fish in freshwater ecosystems. Especially metals, such as copper, can be extremely toxic even at low concentrations. However, due to climate change, aquatic alterations are expected as it is the case of increasing salinity derived from sea level rise, which can also affect aquatic ecosystems equilibrium. Furthermore, during their early development fish suffer several morphological and physiological modifications and thus, it is crucial to make a proper evaluation to investigate how these stress factors can affect the different life stages. Accordingly, this work aimed to assess the sensitivities of different early life stages of Danio rerio to copper and NaCl (to simulate increased salinity). For this, embryos (2h hpf) and larvae (96h hpf) of zebrafish (D. rerio) were exposed to copper sulphate and NaCl and several morphological and functional endpoints were monitored during a 96h period. In the first experiment, embryos were exposed to copper (4.78 - 39.85µM) and NaCl (1.85 - 26.27g/L); in the second experiment, larvae were exposed to the following range concentrations of copper (0.45 - 4.7µM) and NaCl (5 - 8.7g/L). Acute mortality was observed for both exposure to copper and NaCl, however, the larval stage was more sensitive to copper and to NaN3 than embryos. Moreover, in the embryo assay with NaCl, it was only observed significant mortality after the 96h. The protective enclosure of embryos could be one of the major factors contributing for their higher resistance. Even so, both copper and NaCl were responsible for other significant sublethal effects on embryos. Copper had an inhibitory effect on the growth rate and caused a reduced developmental rate and on the other hand, NaCl caused several tail deformities and edemas. These adverse effects on embryos can, as mortality, compromise the long term survival of these organisms. It is important to note that copper was the stress factor where the differences between sensitivity of embryos and larvae were more pronounced. Obtained results suggest that toxicity of copper and NaCl can be very different depending on the life stage. Also, several sublethal effects must be taken into account to better evaluate the real toxic effect of these compounds to zebrafish.

TH 020
Arabian killifish: An indicator model for laboratory environmental risk assessments of the Qatari coastal area

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SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
Fish embryo toxicity (FET) studies can provide an alternative to traditional fish acute toxicity testing. FET methods offer many benefits; they are considered animal alternatives, they require relatively small sample size and reduced time to determine toxicity endpoints. The use of Arabian killifish (Aphanopus dispar) embryos is proposed as an alternative to using juvenile or adult fish for environmental risk studies in the state of Qatar. The Arabian killifish are small fish about (5-6 cm) total length, from the family Atherinidae and breeding behavior, research was initiated in early 2010 to establish a laboratory fish culture capable of producing high quality embryos in sufficient quantity for toxicity studies. Another important objective was to identify the developmental stages for the Arabian killifish and to understand their relative sensitivity to more established FET species. The standard FET approach that uses Zebrasfish (Danio rerio) or Medaka was adapted to the Arabian killifish. Behavioral patterns, feeding optimization, diurnal cycle, male to female ratio as well as other factors were studied in the laboratory. Embryo development was observed and documented from fertilization to hatch. Reference toxicant studies were performed and the results compared to other species. A reproducing laboratory culture was established. Embryo development was studied and the key development stages were identified. The results of the reference toxicant testing confirmed similar sensitivity to toxicants as to other species. We have been successful in the production of quality embryos for testing, both in terms of viability (fertilization) and quantity released. The developmental stages of the Arabian killifish have been identified to provide an array of potential endpoints for ecotoxicology studies. Organism sensitivity appears to be within the desired range for vertebrate species. We conclude that the Arabian killifish exhibits the traits needed for successful FET testing and that they have utility as a vertebrate indicator species.

TH 021

Exploring the relationship between initial test medium concentrations and accumulation of organic chemicals in cells/tissues in in vitro toxicity experiments using an equilibrium partitioning model

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Reliable toxicity data are a crucial requirement for conducting hazard/risk assessment of contaminants in the environment. However, data are limited or non-existent for a large number of contaminants of potential concern (1). Because of the high cost, time requirements and ethical considerations surrounding in vivo animal experiments, it is quite difficult to validate the data. However, under certain circumstances, use of in vitro data will play a significant role in the promotion of this promising area of research. It has been shown that in vitro data can almost always provide important mechanistic insight (e.g. mode of action), direct use of such data for assessing the toxicological implications of measured contaminant levels in humans and wildlife require that cellular concentrations be characterized (as opposed to the initial test medium concentrations, which are typically reported). Several in vitro studies have noted that measured cellular concentrations of hydrophobic chemicals are substantially higher than the initial test medium concentrations and stress the importance of relating external to internal levels (4,7). These findings can be rationalized in the context of the phenomenon of bioconcentration, whereby organic chemicals partition preferentially into biological phases (such as lipids) at the expense of the aqueous phase. Other partitioning behaviours of potential relevance in the context of an in vitro toxicity test include sorption to the vessel walls, volatilization into headspace and interactions with serum constituents (e.g. proteins) or other dissolved organic matter. To explore the behaviour of organic chemicals in such test systems, these sorption relationships were integrated into a balance and equilibrium partitioning model which was then applied to a generic test system for chemicals covering a range of physical-chemical property values. Key model outputs include mass distribution, cellular concentrations, aqueous phase concentrations (in comparison to water solubility limit) and external/internal concentration enhancement factors as a function of hydrophobicity. The influence of assumptions regarding the affinity of cells for chemicals (i.e. sorption capacity), and mass of cells/volume of aqueous phase were also explored. While the results are illustrative in nature (i.e. cannot be directly extrapolated to any particular study), they provide useful insights into experimental design (e.g. how to avoid super-saturating the aqueous phase) and interpretation of results.

TH 022

Quantification of chemical concentration in multiwell plate-based assays: requirements, possibilities and challenges

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The use of animals for experimental studies, e.g. toxicity testing of chemicals, gives rise to serious ethical concerns. The development of new alternative procedures and models is therefore timely. Among the most promising alternative methods are cell line- and fish embryo-based assays. Both approaches are commonly conducted using multi-well plates to enable high throughput screening. Many replicates and very little testing material is required. However, these formats also come with caveats. Because of the small volume, even minute manipulations within culture wells, such as the addition of a test chemical, can be expected to have a significant impact on the dynamics within the well. The high surface to volume ratio, combined with the open nature of the systems, favors losses of test chemicals due to sorption and/or evaporation, leading to a reduced bioavailability of test chemicals. Until today, concentration of chemical concentrations in culture wells has rarely been done. Although, e.g. the OECD draft guideline for the fish embryo toxicity test requires to check that stable chemical concentrations are maintained during the whole exposure duration. Therefore, we set out to design protocol for samples, sample preparation and chemical analysis applicable to the small well volumes present in multi-well plates for cell line- and embryo-based toxicity testing. These protocol will ensure that the exposure methods are reproducible and the exposure concentration is maintained throughout the experiment. To do this we set out to design a protocol for the determination of aqueous phase concentrations (in comparison to water solubility limit) and external:internal concentration enhancement factors as a function of hydrophobicity. The influence of assumptions regarding the affinity of cells for chemicals (i.e. sorption capacity), and mass of cells/volume of aqueous phase were also explored. While the results are illustrative in nature (i.e. cannot be directly extrapolated to any particular study), they provide useful insights into experimental design (e.g. how to avoid super-saturating the aqueous phase) and interpretation of results.

TH 023

Characterising the bioavailability, toxicity and metabolites of benzo[a]pyrene using animal alternatives bioassay

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In vitro methods is targeted to provide alternatives to animal testing by implementing the 3Rs (reduction, refinement and replacement). Several in vitro methods have proved to be versatile tools for toxicity assessment and have shown a potential for high throughput screening of chemicals. Primary hepatocytes isolated from livers are commonly used for uptake and are being used for testing of the bioaccumulation properties of chemicals due to retaining innate cellular properties such as toxicity targets and biotransformation capability. Although the cells may represent alternatives to use of whole animals in some respects, culturing and exposure of cells in high-throughput formats devoid of the natural toxicokinetics and partition behaviour may introduce experimental artefacts giving rise to deviations from in vivo conditions. This deviation could be profound if the compounds have a low aqueous solubility and require metabolic activation (bioactivation) which today represent challenges to in vitro toxicity assessment. The aim of the present work was to assess the role of bioassay conditions on the bioavailability and toxicity of benzo[a]pyrene (BaP) in a primary culture of rainbow trout (Oncorhynchus mykiss) hepatocytes. Assessment of cytotoxicity and classical biomarkers such as EROD induction and CYP1A gene and protein expression was used by im munostaining. In vitro microcopy served to prove the state of differentiation of the cells cultured according to different protocols. Results. Cells were found to build up a multilayer when seeded at high densities (446429 cells/cm2) while seeding of low densities (62500 cells/cm2) led to the desired
DNA repair capacities in fish cell lines RT-W1 and RTGill-W1 and application of a base excision repair comet assay

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Invertebrate fish cell lines are possibly alternative to in vivo fish testing, and new toxicity endpoints such as genotoxicity deserve to be considered in these models. However fish cell lines are suspected to possess low DNA repair activities, which could influence their sensitivity in genotoxicity testing.

The aim of this study was to evaluate in RT-L1-W1 and RTGill-W1 cell lines, the repair kinetics of alkylated damage following exposure to methyl methane sulfonate and recovery with or without the base excision repair (BER) inducer as TCDD (1,10,50 pM) and to two dilution of tested extracts (1,10 mg/ml for P and 0,5, 5 mg/ml for NP). EROD activities were measured in cells exposed for 24 h to the 3-methylcholanthrene (3MC) and to two dilution of tested extracts (in DMSO; max 1%). EROD TEQ values were also calculated. TCDD determined a dose-dependent up-regulation of cyp1a gene transcription 15-folds respect to controls at 10pM and 109-folds respect to control at 50pM, thus confirming the sensitivity of the adopted model. A significant up-regulation of cyp1a was observed only at the highest concentration for the purified fraction of both samples determined a significant dose dependent up-regulation of cyp1a from 2.4 to 15-folds up-regulation for sample A and from 2.1 to 13-folds for sample B. Concerning EROD activity the EC50 measured for TCDD was 17.96 pM. A similar trend of EROD induction has been observed for \( \text{NP} \) and \( \text{BP} \), while no modulation of EROD by \( \text{A} \) or \( \text{B} \) was observed and only a slight induction was observed for \( \text{BP} \) (maximum of 7.4 pmol/mgprot/min) on \( \text{NP} \). Overall results confirm PLHC-1 cells as highly sensitive model to AHR active compounds as significant effects were observed even at low levels of contaminants. Results from bioassays does not agree with TEQ values calculated based on chemical analysis suggesting that compounds other than PCDDs/PCDFs heavily contribute to CYP1A induction at both gene and activity levels. At low contaminations concentrations EROD activity resulted more sensitive than cyp1a gene.

Use of AhR superinducers for potential improvement in the micro-EROD assay

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The micro-EROD assay is an in vitro reporter system which offers several advantages in comparison to the classical AhR binding assay. In particular, this assay is considered to be more of a functional approach as it can be performed in a high throughput format.

Different in vitro alternatives are available to test the toxicity of compounds. For these assays, different cell types can be used. Cell viability is usually determined after the addition of serum to the medium. The

In vitro kinetics of chlorpromazine in cytotoxicity assays with Caco-2, Balb/c 3T3 and HepaRG cells

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Different in vitro alternative to in vivo testing is available for compounds. For these assays, different cell types can be used. Cell viability is usually determined after the addition of serum to the medium. The
The luminescence bacteria bioassay is a rapid, inexpensive (in contrast of the test on living animals), reliable method to identify toxic substances. Toxicity analysis of the pectin samples were performed, and no toxicity was registered. Among samples examined, beet-derived pectin showed the higher toxifying ability. NOEL value of 0.005% (ADS mass 0.01 wt%) of beet-derived pectin was used.

The assay is based on the inhibition of bacterial luminescence caused by the presence of contaminants in a water sample. Turbidity and color may interfere in the luminescence detection, and water samples must be clear, colorless and transparent. In spite of these disadvantages, the assay is being used worldwide for water quality monitoring.

The nutrient solutions and its references were subsequently used in an algal assay in 100 mL beakers containing solution with a cation exchange resin (+R) as a deposit at the bottom of the beakers.

As expected, water samples showed low toxicity to luminescent bacteria in both systems, but the TOXcontrol® online system can detect peaks of unwanted levels of toxicity in the raw water samples. The system examines every 30 minutes a sample of the flowing water and collects data on its toxic effect to the bacteria. The software can also measure the toxicity of a reference sample containing EC50 values of chemical contaminants, in a similar way as the Microtox® Toxicity Analyzer.

The study design was carried out to verify the results produced by the TOXcontrol system, in comparison with results from the Microtox® Toxicity Analyzer, using samples of several reference substances and also water from the intake of a drinking water treatment plant (DWTP) and also the effluent from a wastewater treatment plant.

In this work we present the results of the study on the performance and reliability of an on-line, continuous biomonitoring water system based on the bacterial luminescence toxicity assay.

The Microtox® Toxicity Analyzer® uses, since the 1980s, luminescent bacteria as test organism and has proven a simple, reliable, and quick method to evaluate the toxicity of aqueous samples.

The TOXcontrol® biomonitoring system® (developed by microloan BV in 2006) uses also luminescent bacteria as the test organism to evaluate on-line, the toxicity of a flowing water sample. The system examines every 30 minutes a sample of the flowing water and collects data on its toxic effect to the bacteria. The software can also measure the toxicity of a reference sample collecting EC50 values of chemical contaminants, in a similar way as the Microtox® Toxicity Analyzer.

In the course of this study, the results of the study on the performance and reliability of the TOXcontrol® system were compared with the results obtained with the Microtox® Toxicity Analyzer, using different water samples.

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concentration in solution of the -R devices decreased 50-58 % of initial values. The Cd uptake by the green algae was two times higher in the chloride -R treatment than in its reference solution. The metal uptake in the cells in the +R treatments was unaffected, despite a larger total Cd concentration in solution in the ligand containing solutions. Since several years we have studied the avoidance effect caused by chemical contaminants in soil to earthworms and springtails, with the aim of developing a quick and practical assay to screen large numbers of potentially contaminated soil samples capable to support decision taking on soil quality and remediation practices. The avoidance test proposed presents a promising strategy to improve the efficiency of the soil organisms after exposure to contaminants (see for example ISO 17512-2:2011). This endpoint does not imply mortality or reproduction inhibition, and collembolans are organisms widely present in all territories. This test can provide toxicity results based on the behavior of test organisms after exposure times from 2 to 48 hours. Recently, the avoidance test with collembolans has been reported for registration of chemical substances. On the other hand, classical toxicity tests require exposition of the suspected soil to soil-dwelling organisms for a period of time to assess the biological impact of contaminants. Reproduction and mortality have been the effects studied on earthworms, springtails and other organisms as indicators of soil health. Most of the standard assays call for an exposure time of several days (from 48 hours to 21 days), but regulators require quick tests that quickly assess the quality of soils allegedly contaminated enabling them to take correct decisions. The behavior of soil organisms exposed to chemical contaminants in soil is used as the end point in the avoidance test. Soil organisms move away from contaminated soil and the movement relates to the amount of pollution in the soil samples.

TH 036 Effects of heavy metals on free-living nematodes: a multifaceted approach using growth, reproduction and behavioral assays
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Soil organisms play a key role in soil health. Most of the standard assays call for an exposure time of several days (from 48 hours to 21 days), but regulators require quick tests that quickly assess the quality of soils allegedly contaminated enabling them to take correct decisions. The behavior of soil organisms exposed to chemical contaminants in soil is used as the end point in the avoidance test. Soil organisms move away from contaminated soil and the movement relates to the amount of pollution in the soil samples.

TH 037 Screening of soils using an avoidance assay with springtails
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The behavior of soil organisms exposed to chemical contaminants in soil is used as the end point in the avoidance test. Soil organisms move away from contaminated soil and the movement relates to the amount of pollution in the soil samples. Since several years we have studied the avoidance effect caused by chemical contaminants in soil to earthworms and springtails, with the aim of developing a quick and practical assay to screen large numbers of potentially contaminated soil samples capable to support decision taking on soil quality and remediation practices. The avoidance test proposed presents a promising strategy to improve the efficiency of the soil organisms after exposure to contaminants (see for example ISO 17512-2:2011). This endpoint does not imply mortality or reproduction inhibition, and collembolans are organisms widely present in all territories. This test can provide toxicity results based on the behavior of test organisms after exposure times from 2 to 48 hours. Recently, the avoidance test with collembolans has been reported for registration of chemical substances. On the other hand, classical toxicity tests require exposition of the suspected soil to soil-dwelling organisms for a period of time to assess the biological impact of contaminants. Reproduction and mortality have been the effects studied on earthworms, springtails and other organisms as indicators of soil health. Most of the standard assays call for an exposure time of several days (from 48 hours to 21 days), but regulators require quick tests that quickly assess the quality of soils allegedly contaminated enabling them to take correct decisions.

This work was supported by the “The Eco-technopia 21 project”of Korean Ministry of Environment through and by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology (2011-0003214)
In recent years, the concept of integrated testing strategies (ITS) has been developed as a practical approach toward the 3Rs goals of reducing, refining and replacing animal testing for the toxicological and ecotoxicological assessment of industrial chemicals under the European Directive REACH. The goal is to combine complementary non-animal (in chemico, in vitro, omics) and non-test (in silico) methods with a strong emphasis on mechanisms of action, thus compensating for the reduction in adverse outcome pathways that may arise from individual alternative methods. In this context, organic electrophiles represent a prominent group of particularly toxic contaminants that cover various classes of chemical substances, and may also be generated in vivo through metabolic processes. Their high intrinsic toxicological potential results from their ability to deplete the anti-oxidant glutathione, and to undergo chemical reactions with nucleophilic sites of proteins and the DNA. In this study, a simple in vitro approach is presented that is capable of detecting the reactivity of electrophiles toward the glutathione S-transferase activity of the 50% growth inhibition (EC50) of the unicellular ciliate Tetrahymena pyriformis of a given compound is determined and compared with the corresponding narcosis-level toxicity of an isohydrophobic counterpart. The resultant toxicity enhancement T(H) derived as ratio of narcosis-level over actual EC50 informs about the lack (T(H) > 10) or presence (T(H) ≤ 10) of reactive mechanisms as major determinant of the observed toxicity. This end point can be used to derive structural alerts for a predictive toxicity assessment. Moreover, different mechanisms of reactive toxicity may translate into different level ranges of the toxicity enhancement, thus providing pertinent information in the context of ITS schemes. The approach is illustrated with results for quinones and hydroquinones as two compound classes that may generate reactive oxygen species (ROS) through redox cycling, and -without (quinone) or after metabolic oxidation of quinone groups (hydroquinone). The intended end points are functional alterations to cellular ecosystems, such as de novo fatty acid biosynthesis, and the ROS-mediation of cell death. This work was financially supported by the European Union project OSIRIS (Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Test and Testing Information), contract no. GOC-CT-2007-037017.

TH 042

Predictive assessment of the aquatic toxicity of Michael acceptors using a kinetic chemosensor with 4-nitrobenzenethiol (NBT)


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In aquatic toxicity, the well-accepted narcosis paradigm states that every chemical substance exerts a minimum toxicity proportional to its hydrophobicity. Accordingly, determination of relevant species at risk and how they can be linked to potential exposure in agricultural practice is generally behind. In line with results, the key features with the highest score and also considered for the development of a chronic toxicity test of organic electrophiles is to measure their reactivity toward model nucleophiles, employing so-called chemoassays (in chemico). In this work, 4-nitrobenzenethiol (NBT) is taken as an endogenous thiol group. It has the advantage to be directly detectable by UV-Vis absorption, enabling a fast and low-cost quantification of the toxicologically relevant reactivity. Using this approach, second-order reaction rate constants of reaction with NBT, kobs, were determined for a number of α,ω-unsaturated carbonyls (Michael acceptors). These electrophiles are able to react irreversibly with nucleophilic groups through a Michael-type addition mechanism, and therefore the potential to exert substantial toxicity exists. Our results demonstrate a good correlation between log kobs and the extent of the reactivity (the latter of which was derived from in vivo results of the growth inhibition bioassay with the ciliates Tetrahymena pyriformis).

The NBT chemosensor is found to be a simple and promising tool for the predictive assessment of the aquatic toxicity of organic electrophiles.

TH 043

Linking high-throughput In Vitro Screens with EDSP assays: the causal relationship challenge

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One step in complying with Test Orders under the EU EPAs Endpoint Disruptor Screening Program was compilation of Other Scientifically Relevant Information (OSRI). The EDSP assay was evaluated to determine what requirements were met by existing data and, which of the 11 Tier 1 Endocrine Disruptor Screening Battery (ESB) assays were needed. Many of the List 1 chemicals under ESB Test Orders were analyzed in the EPA’s ToxCast Program, hence ToxCast data were submitted as OSRI in lieu of conducting many similar ESB in vitro assays, and to help support waivers for some in vivo assays. ToxCast seeks to predict human toxicity by measuring the activity of compounds to a set of highly diverse, high-throughput model. The concept presumes that these assays can identify a chemical’s ability to activate so-called ‘toxicity pathways’. In evaluating acceptability of OSRI, EPA rejected ToxCast results claiming that the reliability, responsiveness and relevance of ToxCast were undetermined and that ToxCast does not evaluate all known endocrine targets. In rejecting ToxCast data, EPA claimed its validation status was inferior to that of the individual ESB assays. However, even the predictive value of the in vitro ESB assays for in vivo endocrine activity is still unclear at this time. Although ToxCast data was rejected as OSRI for the ESB, ToxCast data was used to assess and deem acceptable the risks of oil dispersants used for the Deep Water Horizon spill. These decisions have significant implications for the use of screening assays in regulatory modes, yet inconsistencies in application confound what those implications will be.

TH 044

Study of key characteristics of ciliates for development of chronic toxic inactivation test


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The multiassay approach is useful for comparing different conditions and the results are normally oriented to the operational decisions. The development of methods that integrate environmental risks and detection of compounds that cause deleterious effects to living organisms in an area at low cost, reproducibility, ease of implementation and the avoidance of the presence of unmanageable development and minimize impacts to the ecosystems of developing countries.

This study is aimed at developing a multiassay toxicity analysis to define key characteristics to design a method for chronic toxicological evaluation considering aspects of the complete life cycle of organisms, as well as the inherent effects in tropical countries. A multiassay matrix was prepared based on Quality Function Deployment model with each test for consideration the interrelationships between them. The criteria to be used in the matrix factors for assessing the response time, technical difficulty, cost, reproducibility and number of citations in the literature were defined. The total score was calculated by the sum of scores, multiplied by the corresponding weights. The criteria technical difficulty is of great importance in this study as being proposed for use in developing countries, where technological development is generally behind. In line with results, the key features of the highest score and also considered for the development of a chronic toxicity test were reproduction, mortality, time to first reproduction and total size of the organisms.

TH 045

Etocotoxicity of amphibian and reptiles. Novel approaches for linking contaminant effects with population declines

O. Korner, N. Lutzmann, J.D. Ludwigs, G. Vogel, C. Dietzen

RIFCon GmbH, Hirschberg, Germany

Relevant data requirements under the EU regulation 1107/2009, reptiles come into question regarding risk assessments of plant protection products (PP). Although no specific data requirements on reptiles are stipulated in the respective EU documents (e.g. SANCO 11802/2010/rev July 2010) some toxicity data are available in the open literature. These data are used to be intended in the risk assessment. One potential approach for reptile risk assessment may be the way how it is subsequently established and translated into consideration the interrelationships with the environment. A detailed literature survey on habitat use and the biology of lizards occurring in North and Central Europe (geographical zones according to SANCO/6896/2009 rev 1) was conducted. The study focused on the habitat use of the different species, but also evaluates the different diets as a source of data. The obtained data may allow the determination of relevant species at risk and how to be linked to potential exposure in agricultural practice.

TH 046

Relevant lizard species for the risk assessment of plant protection products in Northern and Central Europe

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According to the new data requirements under the EU regulation 1107/2009, reptiles come into question regarding risk assessments of plant protection products (PP). Although no specific data requirements on reptiles are stipulated in the respective EU documents (e.g. SANCO 11802/2010/rev July 2010) some toxicity data are available in the open literature. These data are used to be intended in the risk assessment. One potential approach for reptile risk assessment may be the way how it is subsequently established and translated into consideration the interrelationships with the environment. A detailed literature survey on habitat use and the biology of lizards occurring in North and Central Europe (geographical zones according to SANCO/6896/2009 rev 1) was conducted. The study focused on the habitat use of the different species, but also evaluates the different diets as a source of data. The obtained data may allow the determination of relevant species at risk and how to be linked to potential exposure in agricultural practice.
The study focused on the habitat use of the different species, but also evaluates the different diets as a source of exposure. The data obtained may allow the determination of relevant species at risk and how they can be linked to potential exposure in agricultural practice.

TH 052
Are the Mediterranean chameleon species possible focal species for risk assessments of PPPs in Europe? N. Lutzmann, G. Vogel, W. Böhme
RIPCORN GmbH, Hirschberg, Germany
According to the new data requirements under the EU regulation 1107/2009, reptiles come into question regarding risk assessments of plant protection products (PPP). Although no specific data requirements on reptiles are stipulated in the respective EU documents (e.g. SANCO 11802/2010 rev July 2010) some toxicity data are available in the open literature. These data are intended to be used in the risk assessment. One potential approach for reptile risk assessment may be the way how it is successfully established for birds and mammals. The major routes of exposure in that case are diet and drinking water. In order to provide a scientific basis for reptile risk assessment, a detailed literature survey on habitat use and the biology of lizards occurring in South Europe (geographical zones according to SANCO/6896/2009 rev 1) was conducted. The study focused on the habitat use of the different species, but also evaluates the different diets as a source of exposure. The data obtained may allow the determination of relevant species at risk and how they can be linked to potential exposure in agricultural practice.

TH 053
Relevant snake species for the risk assessment of plant protection products in Southern Europe N. Lutzmann, C. Dietzen, J. Gerlach, O. Körner, G. Vogel
RIPCORN GmbH, Hirschberg, Germany
According to the new data requirements under the EU regulation 1107/2009, reptiles come into question regarding risk assessments of plant protection products (PPP). Although no specific data requirements on reptiles are stipulated in the respective EU documents (e.g. SANCO 11802/2010 rev July 2010) some toxicity data are available in the open literature. These data are intended to be used in the risk assessment. One potential approach for reptile risk assessment may be the way how it is successfully established for birds and mammals. The major routes of exposure in that case are diet and drinking water. In order to provide a scientific basis for reptile risk assessment, a detailed literature survey on habitat use and the biology of lizards occurring in South Europe (geographical zones according to SANCO/6896/2009 rev 1) was conducted. The study focused on the habitat use of the different species, but also evaluates the different diets as a source of exposure. The data obtained may allow the determination of relevant species at risk and how they can be linked to potential exposure in agricultural practice.

TH 054
Plant protection products and juvenile amphibians: no matter of concern or unexpected risk? T.W. Schmidt, A. Alschier, S. Hoeger, S. Pieper, C. Bruehl
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In order to fill the data gap of the potential risk of plant protection products on the terrestrial life stage of amphibians, approx. 1-2 months old juveniles of the Common Frog (Rana temporaria) were caught in the field and exposed to six different plant protection products in the laboratory on bare soil for one week. For each plant protection product three application rates, including the practically used field application rate, were selected to determine the LR50. Our results show that the majority of the plant protection products used in agriculture are not expected to cause any adverse effects on the studied species. Especially the use of neonicotinoid products should be further investigated in order to determine potential risks for native amphibians.

TH 055
Assessing atrazine toxicity to the Australian striped marsh frog, Limnodynastes peronii K.A. Siddiqua, S.P. Wilson, R. Alquezar
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Visita Australia Queensland, Gladstone, Australia
Abstract
Atrazine is one of the most extensively applied herbicides in Australia and frequently detected in surface and ground water samples. In overseas studies, it has been reported that atrazine can cause reproductive and developmental abnormalities in exposed amphibians. Limited studies have been carried out in Australia, resulting in subsequent gaps in knowledge particularly among Australian species. To explore the effects of atrazine to native frogs, the striped marsh frog, Limnodynastes peronii, was exposed to a range of plant protection products, since they migrate through agricultural fields either from their larval aquatic habitats to over-wintering sites in autumn or back in spring for egg-laying.

TH 056
Toxicity of eucalypt and pine leaf leachates in zebrafish (Danio rerio) and iborian frog (Pelophylax perezi) early-life stages J. Medeiros, K. Monaghan, A.M.V.M. Soares, M.S. Monteiro
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University of Aveiro & CESAM, Aveiro, Portugal
CESAM, Dep Biologia, Universidade de Aveiro, Aveiro, Portugal
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Abstract
The toxicities of eucalypt and pine leaf leachates in zebrafish (Danio rerio) and iborian frog (Pelophylax perezi) early-life stages were investigated in the present study. The toxicities of eucalypt and pine leaf leachates were determined by using the fish embryo teratogenesis assay-Xenopus (FETAX). The endpoints monitored included mortality and sub-lethal parameters in embryonic development such as pericardial oedema, tail deformation and hatching rate. Five concentrations of each leachate were tested, ranging from 0.64 to 20.48 and 1.6 to 12 g L-1 of Eucalyptus leachates and P. perezi, respectively; and from 1.6 to 32 g L-1 of Pine in both species. Simultaneously, the water physico-chemical parameters and phenolic content were evaluated in eucalypt and pine leachates. In general, leachates were found to reduce water dissolved oxygen and pH, and increase dissolved phenolics and metal concentrations. The highest toxic effects of eucalypt leachates for zebrafish (LC50 h = 3.32 and 12.25 g L-1 of eucalypt and pine leachates, respectively), while for embryo frogs the different leachates presented similar toxicity (LC50 96 h = 14.97 and 13.26 g L-1 of eucalypt and pine leachates, respectively). The embryonic developmental alterations registered in both species will be presented and discussed considering the type of leaf leachate tested and their respective water-physico-chemical parameter characteristics and phenolic content. These data demonstrate that leachate toxicity may contribute to the impact of pine and eucalypt plantations on aquatic biodiversity.

TH 057
Alterations of embryonic development and mRNA expression in Xenopus laevis caused by perfluorooctane sulfonate (PFOS) L. San Segundo, L. Guimaraes, G. Carbonell, C. Fernandez, L. Guilhemino, M.V. Pablos
CESAM, Dep Biologia, Universidade de Aveiro, Aveiro, Portugal
Portugal
Abstract
Perfluorooctane sulfonate (PFOS) is a persistent, bioaccumulative and toxic substance, as demonstrated by its propensities to negatively impact on various species of animals at all trophic levels. In the present study, the toxicity of eucalypt and pine leaf leachates that may be particularly damaging in intermitting streams, was evaluated using embryo toxicity testing with Danio rerio, a tropical cyprinid used in toxicological research, based on the OECD draft guideline on Fish Embryo Toxicity (FETAX) and with the iborian frog Pelophylax perezi based on the ASTM guideline on Frog Embryo Teratogenesis Assay-Xenopus (FETAX). The endpoints monitored included mortality and sub-lethal parameters in embryonic development such as pericardial oedema, tail deformation and hatching rate. Five concentrations of each leachate were tested, ranging from 0.64 to 20.48 and 1.6 to 12 g L-1 of Eucalyptus leachates and P. perezi, respectively; and from 1.6 to 32 g L-1 of Pine in both species. Simultaneously, the water physico-chemical parameters and phenolic content were evaluated in eucalypt and pine leachates. In general, leachates were found to reduce water dissolved oxygen and pH, and increase dissolved phenolics and metal concentrations. The highest toxic effects of eucalypt leachates for zebrafish (LC50 h = 3.32 and 12.25 g L-1 of eucalypt and pine leachates, respectively), while for embryo frogs the different leachates presented similar toxicity (LC50 96 h = 14.97 and 13.26 g L-1 of eucalypt and pine leachates, respectively). The embryonic developmental alterations registered in both species will be presented and discussed considering the type of leaf leachate tested and their respective water-physico-chemical parameter characteristics and phenolic content. These data demonstrate that leachate toxicity may contribute to the impact of pine and eucalypt plantations on aquatic biodiversity.
This work has been funded by CSD2006-00044 -CONSOLIDER- and RTA2010-00004-C02-01 projects. LSS acknowledges her FPI-INIA fellowship.

TH 058
Assessment of potential toxicity of wastewater treatment plant effluents in a Xenopus laevis embryo test using gene expressions as early response biomarkers

L. San Segundo, F. Martini, G. Carbonell, C. Fernandez, M.V. Pablos
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TH 059
Effects of larval estrogen exposure on adult reproductive potential in the amphibian model, Silurana tropicalis

B. Pickford, S. Larroze
University of Siena, Siena, Italy

TH 060
Exposure to cadmium: evaluation of the effects on the larval epidermis of Lithobates catesbeianus

C.S. Almeida, L. Costa, M. F. Rodrigues
Universidade Federal de São Carlos, Sorocaba, Brazil

TH 061
Intercellular assessment of the effects posed by historical exposure to chemical contamination in natural populations of the Perez's frog, Pelophylax perezi (Seoane, 1885)

1, 2, 3, 4, 5

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3, 4
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Departamento de Biologia, Universidade do Porto, Porto, Portugal

TH 062
Development of new non-destructive biomarkers in the loggerhead sea turtle: first evidence of CYP1A in skin biopsy slices treated with PAhS, Ocs and PBDEs

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Inhibition of CYP1A has been proven by a large number of studies to be a sensitive and specific biomarker of exposure to some classes of lipophilic contaminants. However,
The use of this biomarker has been mostly destructive, since the liver is the main target organ for analysis. This study is part of a larger project aimed at developing non-invasive biomarkers in the endangered species of C. caretta in the Mediterranean. In particular it focuses on developing and validating the induction of CYP1A as a non-invasive biomarker of exposure in skin biopsy slices during the first part of the study, the Western blot method to evaluate protein expression of CYP1A in this species in invasive biomarker of exposure in skin biopsy slices. During the first part of the study, the Western blot method to evaluate protein expression of CYP1A in this species.

**TH 063**

**Effects of lead shot pellets contamination on oxidative stress biomarkers and plasma biochemistry in turtles**

M. Martínez-Haro1, A. J. Green2, A. M. V. M. Soares3

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**Abstract**

The aim of this project addresses the question: Could be that changes in environmental temperature regimes, due to climate changes, influence the adverse effects caused by contaminants to several amphibian species have been performed at moderate, constant ambient temperature of approximately 20 ºC, which is an over-simplification of what actually happens in the environment for example, temperatures as high as 40 ºC may occur, with diurnal variations of 5 ºC. In fact, there is fragmental but strong evidence suggesting that temperature induces changes in adverse effects of chemicals, hence, the effect under laboratory conditions may substantially differ from that observed in real ecosystems.

The aim of this project, addresses the question: Could be that changes in environmental temperature regimes, due to climate changes, influence the adverse effects caused by pesticides on amphibians? Future results from small scale experiments (laboratory experiments) may be validated by conducting studies at large spatial and temporal scales with a higher ecological realism (mesocosms). The underlying mechanisms responsible for the changes observed in experimental systems at a large scale, not replicable, may be examined at lower levels, such as subcellular levels (biomolecular interactions), individual level (survival, growth/development, behaviour) or population level (recruitment).
The Arctic is an ideal indicator region for Persistent Organic Pollutants (POPs) because it is far from sources of these contaminants. To reach remote areas such as the arctic, POPs undergo long range transport via multiple cycles of atmospheric deposition and re-volatilization, known as the grasshopper effect. Thus, atmospheric deposition is believed to be an important pathway for arctic contamination. The aim of this study was to quantify the flux of legacy POPs from atmosphere to land in the Arctic as well as screen for emerging POPs new to the arctic on i) partitioning properties, ii) compartment (e.g. air, water, soil) and iii) stage of emission (uptake vs. depuration).

**TH 074**
Emerging persistent organic pollutants in arctic air, surface water and snow in Ny-Alesund, Svalbard
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Emerging persistent organic pollutants (ePOP) may reach ecologically sensitive Arctic environment via atmospheric and/or oceanic long range transport. They are subject to a variety of processes in the Arctic environment such as degradation, bioaccumulation and interaction between the atmosphere, snow and water. Additionally, climate change may significantly influence the transport and environment fate of ePOPs in the Arctic. As a part of collaborative German-French program at joint French-German Arctic Research Base (AWIPEV) in Ny-Alesund, Svalbard, this project is proposed to investigate the occurrence and long term trends of ePOPs in arctic air, water and snow.

High-integrated volume air samples were taken on the roof of IPEV station using a high-volume pump operated for 7 days to obtain a volume of ~2000 m³. A glass fiber filter is used to trap the airborne particles and the gaseous contaminants are collected with a PUF/XAD-2 resin column. Surface snow samples were collected on the glaciers in Ny-Ålesund and seawater samples were obtained in Kongsfjorden in May of 2011. All samples were analyzed for neutral and ionically fluorinated compounds, brominated flame retardants, dechlorane plus and current-use pesticides. Data achieved from this study may improve models to predict the environmental progression and assess the effect of climate change on the long-range transport and the fate of the ePOPs in the Arctic ecosystem.

**TH 075**
Brominated flame retardants in ice cores from Svalbard: an east-west comparison
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1University Center on Svalbard, Longyearbyen, Norway
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Atmospheric deposition of legacy and emerging persistent organic pollutants in Northern Sweden
S.R. Newton1, T.E. Budiman1, M. Tyskling1, K. Wiberg1
1Swedish University of Agricultural Sciences, Uppsala, Sweden

The Arctic is an ideal indicator region for Persistent Organic Pollutants (POPs) because it is far from sources of these contaminants. To reach remote areas such as the arctic, POPs undergo long range transport via multiple cycles of atmospheric deposition and re-volatilization, known as the grasshopper effect. Thus, atmospheric deposition is believed to be an important pathway for arctic contamination. The aim of this study was to quantify the flux of legacy POPs from atmosphere to land in the Arctic as well as screen for emerging POPs new to the arctic on i) partitioning properties, ii) compartment (e.g. air, water, soil) and iii) stage of emission (uptake vs. depuration).

**TH 076**
Atmospheric deposition of legacy and emerging persistent organic pollutants in Northern Sweden
S.R. Newton1, T.E. Budiman1, M. Tyskling1, K. Wiberg1
1Swedish University of Agricultural Sciences, Uppsala, Sweden

**TH 077**
Assessing the potential influence of global climate change on the long-range transport and accumulation of organic contaminants in the Arctic environment in relation to physical-chemical properties
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The potential influence of global climate change (GCC) on the fate, transport, bioavailability and bioaccumulation of organic contaminants is rapidly becoming an area of interest for scientists engaged in environmental and toxicology research. The Arctic region is of particular concern due to changes to the cryosphere that are already affecting communities living in the Arctic and that are expected to continue to occur in this region in the future and finally that human inhabitants of this region harvesting local food resources (e.g. seals) have an elevated exposure to many organic contaminants of concern (i.e. POPs) (1-3). Global climate change could potentially influence exposure to organic contaminants via direct (e.g. increased water temperature and altered fate in aquatic environments), indirect (e.g. lifestyle transition) or both changes in the environment (the focus of this paper) that cause changes in the environment (reduced sea-ice + increased delivery of imported food items) could also be highly influential in the long-term (4). The purpose of this investigation is to explore the potential influence of changes to the physical environment (e.g. temperature, precipitation rate, etc.) on the local organic transport and accumulation (productivity) of the local population (i.e. the local community). Simulations were conducted for hypothetical chemicals covering a wide range of partitioning properties for a 20-year constant emission period followed by a 20-year depuration (i.e. zero primary emission) period. While sensitivities to the GCC scenario parameterizations considered here are relatively low (typically within a factor of two), response for emerging POPs new to the arctic on i) partitioning properties, ii) compartment (e.g. air, water, soil) and iii) stage of emission (uptake vs. depuration).
Increasing environmental concentrations of POPs in the Arctic in a future warmer climate

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The Danish Eulierian Hemispheric Model (DEHM) was applied to study the impact of climate change on contaminant levels in the Arctic. DEHM is a 3-D atmospheric chemistry-transport model developed over the last 20 years to study the transport of SO2, SO4, and Pb to the Arctic. All important source regions for the Arctic are included in the model domain, which covers the Northern Hemisphere. This model has been developed further to include mercury species/chemistry, and Persistent Organic Pollutants (POPs).

The model system has a spatially detailed 3-D atmosphere up to 15 km over the surface. In addition, it has four surface compartments: a 75 m thick ocean layer, a 15 cm thick snowpack, a seasonal snowpack, and a 15 cm thick soil layer, simulating vegetation and the seasonal snowpack.

The model system has been run with meteorology obtained from ECHAM5/SP-OIM (SRES A1B scenario) for two different decades: 1990-1999 and 2090-2099. In this climate scenario the global temperature is predicted to increase continually with 3 ºC by the end of 2100 relative to the period 1971-2000.

The development of gas fields on the Yamal Peninsula (West Siberian Arctic) leads to disturbances of landscapes. Vegetation of the Arctic tundra is the most changeable layer, a 15 cm thick soil layer, and dynamically evolving vegetation and seasonal snowpack compartments.

This presentation will emphasize the TF HTAP integrated approach to measurements, modeling, emissions and impacts, but focus on measurements and modeling, with special attention to climate change, which may alter POPs cycling between environmental compartments. Most projected projects are not coordinated in a way to facilitate

Concentrations and potential sources of some semi-volatile organic contaminants in the air at Little Fox Lake, Yukon Territory, Canada

J.N. Westgate¹, U.M. Sofowote², P. Roach³, P. Fellin¹, J. Sáiz, E. Sverko⁴, Y. Su⁴, F. Wania⁴, H. Hung⁴
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Environment Canada, Burlington, Canada

Heavy metals and nutrients in soils of the Yamal Peninsula (West Siberia) as indicator of ecosystem stability

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The development of gas fields on the Yamal Peninsula (West Siberian Arctic) leads to disturbances of landscapes. The vegetation of the Arctic tundra is the most changeable layer, a 15 cm thick soil layer, and dynamically evolving vegetation and seasonal snowpack compartments.

The patterns of vegetation and recovery depend directly on soil chemistry. Vegetation on soils depleted in major and trace elements is poor, and such landscapes are weakly sustainable. Thus, the sustainability of plant-soil systems largely depends on their chemical composition.


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The development of gas fields on the Yamal Peninsula (West Siberian Arctic) leads to disturbances of landscapes. Vegetation of the Arctic tundra is the most changeable layer, a 15 cm thick soil layer, and dynamically evolving vegetation and seasonal snowpack compartments.

This presentation will emphasize the TF HTAP integrated approach to measurements, modeling, emissions and impacts, but focus on measurements and modeling, with special attention to climate change, which may alter POPs cycling between environmental compartments. Most projected projects are not coordinated in a way to facilitate
synergy and integration. The TF HTAP collaboration enabled communication between specialists and identification of the most important cross-dependencies and research gaps. Furthermore, the review permitted the collating and sharing of an immense amount of data, among which recent IPY observations played a crucial role. Modeling enabled comparison between diverse and, sometimes, competing approaches with the benefit that model parameterizations could be exchanged. This intercomparison led to better understanding of the vastness of models and the importance of model intercomparisons.

Mindful of overlaps with other international organizations, the TF HTAP is initiating a new four-year work plan with a multi-pollutant-co-benefit approach that harmonizes experiments, model parameterization and evaluation of results. Collaborations are voluntary, and key areas, like global POPs emission estimates, remain bottlenecked for further progress. The TF HTAP has already provided a platform for experts from many countries in a number of POPs, and the knowledge gained will be useful to inform future regional and international research and mitigation strategies.

POP concentrations in Arctic biota

Persistent organic pollutants (POPs) have been used extensively in industry, agriculture and consumer products and have reached the Arctic via atmospheric and ocean transport. They include organochlorine compounds such as polychlorinated biphenyls (PCBs) and DDT, which are regulated by the Stockholm Convention and were banned in 2001. Other organochlorines (e.g., DDE) are still important pollutants in Arctic marine systems. The most important persistent POPs are the polybrominated diphenyl ethers (PBDEs) and the polybrominated biphenyls (PCBs). The Arctic is a pristine area which is less affected by local pollution sources compared to other parts of the world, and is therefore a reference to study the transport and fate of these pollutants.

Transport and fate of POPs

Persistent POPs can be transported long distances and can accumulate in organisms and ecosystems. The levels in Arctic biota are influenced by both direct inputs from the atmosphere and ocean transport. As a result, the Arctic has high levels of POPs, which can reach remote areas and have significant ecological impacts. For example, POPs in the Arctic have been linked to changes in the reproductive success of marine mammals and birds, and to changes in the growth and development of polar bears.

Impact of POPs on ecosystem function

POP exposure has been linked to changes in the function of Arctic ecosystems. For example, POPs have been found to affect the steroid hormone levels in polar bears, which can affect their reproductive success. POPs have also been linked to changes in the nutrient cycling and energy flow in the Arctic food web. These changes can have cascading effects on other trophic levels and can affect the stability and productivity of the ecosystem.
Impact of dense shelf water cascading in the transfer of organohalogenated contaminants offshore Svalbard, Arctic Ocean

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Legacy Organochlorine compounds including Polychlorinated Biphenyls (PCBs), Hexachlorobenzene (HCB) and Hexachlorocyclohexane (HCHs) are ubiquitous pollutants that reach the Arctic atmosphere, oceans and sediments. In this work, the authors propose to use the surrounding area of the Svalbard archipelago as a source of influence of organic contaminants to the Antarctic atmosphere, seas and, consequently, to the deep marine environments. This work aims to determine the cascading effects of the Svalbard archipelago on the Antarctic Ocean to help explain the spatial and temporal variability of organochlorines in the marine environment of Antarctic waters. For this purpose, sediments were collected from 30 meters to 4000 meters of water depth in the surroundings of the Svalbard archipelago, and the resulting data were analyzed to determine the potential sources of such compounds and their further influence in the Antarctic region.

The results showed that the presence of organochlorines in the sediments of the Svalbard archipelago is influenced by various factors, including the distance from the source area and the depth of the water column. The authors concluded that the cascading effects of the Svalbard archipelago on the Antarctic Ocean are significant and should be taken into account in further studies on the transport of contaminants in the Arctic and Antarctic regions.
EC03P - In situ remediation of metalloid contaminated soils: exploration of emerging technologies and long-term effects of previously remediated sites

TH 097
Helenius tuberculosis L. grown with multiple pollution in greenhouse conditions
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IMDEA, Madrid, alcalá de henares, Spain

Helenius tuberculosis L. (Compositae) offers wide technological interest as a bio-ethanol due to its high potential for biomass production. Recently studies showed the tolerance of this crop to heavy metals that can no longer be used for agriculture applications. The present work reports data concerning to the tolerance of two cultivar-clones of H. tuberosus (VR and D19) grown in hydroponic culture with a multipolluted solution of heavy metals, in order to evaluate their use as energetic crop in polluted sites. The experiment was carried out in a greenhouse under controlled conditions. Tubers were recollected in field and transferred to plastic trays filled with turf during two weeks. Then the plants were carefully rinsed in distilled water and transferred to hydroponic culture with coarse perlite. Plants were fertilized with a modified Hoagland’s solution and treated with heavy metals: (i) Treatment 1: 30 mg-L-1 of As (V), Cd, Cr (VI) and Ni; (ii) Treatment 2: 30 mg-L-1 of Cu, Zn, Pb and Cd; (iii) Treatment 3: 30 mg-L-1 of As (V), Cd and Ni. Control plants were fertilized only with nutrient solution. The plants were harvested after two weeks, separated into leaves, stems, roots and tubers, dried in an oven at 70°C. The protuberance and root dry weight were significantly reduced in both cultivar-clones as comparison of the control when plants grown in presence of a mixture of As (VI), Cd, Cr (VI) and Ni. Biomass of both cultivar-clones showed no significant differences when treatment with Cu, Zn, Pb and Cd was applied. VR was not affected by As (V), Cd and Ni, while D19 biomass was significantly reduced with this treatment, in the case of leaves and roots by 51% and 55%, respectively. The results illustrate that the metalloids, 2-hydroxy quinoxaline and oxon which are more toxic than the parent compound seem to persist for a longer time. The presence of these metabolites in the concerned biological fluids can help in the identification of quinalphos as the source of poisoning.

TH 098
Numerical modeling of a Phyto-extraction process
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The study of phytoextraction remediation processes involving plants that extract contaminants and accumulate them in plant tissues. Despite its low cost and low impact presents difficulties in estimating the efficiency of the process and the time required for remediation. The results depend on a complex network of interactions between plants, soil and atmosphere in the presence of contaminants. This research aims to estimate the time required for remediation of soil. Pedologic and climatic data from Rio de Janeiro were used. The initial contamination levels were determined based on real cases of industrial activities and waste disposal. The root contaminant uptake model was previously calibrated in three phases: pre-contamination (determination of hydrological conditions and water table), contamination (evolution of the plume of contamination before planting), remediation (remediation process simulation). Although the results were specific to the chosen scenario, it was observed that the efficiency of the phyto-extraction decreased with depth reached by the plume of contamination. The proposed methodology, despite the tools for calibration enhancements and models for vegetation, provided indications that may be useful in the design of phyto-extraction projects.
assessed in batch non-metallic Me-So4 at pH 4.0 accounted for 40600 mg Cu/kg and 39000 mg Cd/kg and was comparable with natural sorbents (e.g. peat). In the environmentally relevant systems, such as ARD generating sites, a number of factors may influence sorption capacity of materials, of these pH, competitive effect of other ions, adsorbent-adsorbatte contact mode, dominating ions or solutes and the temporal or permanent transformation of adsorbent are the most important ones. Sorption experiments taking into account these factors were designed. The experiments were divided into three parts: (a) determination of the adsorption isotherms, (b) determination of the adsorption capacities of the Fe ions at different pH and (c) determination of the adsorption capacities under different conditions. The capacity of the adsorbent to adsorb Fe ions was found to be pH-dependent, with the highest adsorption capacity at pH 4. The adsorption isotherm was found to be of the Langmuir type, indicating monolayer adsorption.

TH 100

Coculture fermentations, instead of single cultivation can be utilized advantageously, in industrial biotechnology in the production of chemicals, particularly during the use of lignocellulosic material as feedstock, since both hydrolysis and fermentation processes can be carried out in the same reactor.

The use of Lactobacillus penusos and Lactobacillus rhamnosus allow biosurfactants from both fractions. Biosurfactants are amphiphilic compounds of microbial origin with a pronounced surface activity and with several advantages over chemical surfactants including lower toxicity, higher biodegradability, and effectiveness at extreme temperatures or pH. Biosurfactants are potential candidates for many commercial applications in the biomedical, petroleum, and food processing industries.

This work deals with the application of biosurfactants derived from different agroindustrial wastes, during cocultures processes, in the bioremediation of soils contaminated with heavy metals.

TH 101
Biosorption of heavy metals from gold mine wastewaters by Penicillium simplicissimum immobilized on zeolite: kinetic, equilibrium and thermodynamic studies H. Tutu, E.N. Bakunala, E.M. Cukrowska, C.J. Slater, I.M. Weiersrey University of the Witwatersrand, Johannesburg, South Africa

A biosorber based on zeolite with both (living and heat-killed fungal biomass) was developed for the clean up of heavy metals from gold mine wastewaters. With an initial concentration of 500 mg l-1 at pH 3 - 4 for a simple component system, 99% adsorption was observed for: Cu2+, Co2+, Cr3+, Fe2+, Ni2+, Zn2+, Hg2+. Immobilisation of fungi on zeolite yielded higher biomass, showing the potential of this study towards remediation of polluted mine sites. Desorption results showed that the adsorbent could be reusable.

TH 102
Mercury immobilization using modified mackinawite as reactive layer in in-situ capping M.R.M. Chaves, P. R. P. Gamberotti1, R.D. Delaune2, P.M. Buchele2 1University of Sao Paulo, Sao paulo, Brazil 2Indiana State University, St paul lez durance, France

Mercury and methyl mercury adversely affect human, birds, and mammals health. The effects of mercury contamination are cause of global concern; thus remediation of contaminated sites is a high priority issue. This study investigated the mercury immobilization using mackinawite modified with L-cysteine as an active layer in in-situ capping. Mackinawite (FeS) is a promise material to be used in in-situ capping due to its excellent capacity for mercury uptake from anoxic contaminated sediments. The surface modification with L-cysteine increases the mackinawite oxidation resistance, becoming more stable in field conditions. Modified mackinawite as a reactive capping layer was studied in lab scale simulator systems using lake sediment contaminated with mercury. The mercury profile towards the cells showed that modified mackinawite is an excellent material to be used as reactive layer in in-situ capping. In this study the capability to immobilize mercury was evaluated. Incubation experiments showed that modified mackinawite has higher mercury methylation inhibition capacity, than the observed from unmodified mackinawite.

TH 103
In situ arsenic and lead stabilization and remediation using green chemistry and nano iron welding fumes B.M. Miller1, E.A. Kirk2, T.L. Todd3, A.Z. Zimmers2 1University of California, Cincinnati, United States of America 2University of Cincinnati, Cincinnati, United States of America 3University of Cincinnati, Cincinnati, United States of America

Arsenic (As) and lead (Pb) are highly toxic naturally occurring elements found in soils worldwide and is recognized as a carcinogenic responsible for neurological, gastrointestinal, cardiovascular, and reproductive illnesses. Toxic concentrations of As and Pb pose a public health threat particularly to children because of their tendency to consume soil via hand-to-mouth contact. A recent study by Byers and Ryan (2010) showed that As concentrations in soils increased by 200% from a 1.5 m depth. Remediation efforts that sequester As and Pb within recalcitrant occluded Fe minerals while controlling soil physiochemical factors could give risk assessors and stakeholders confidence that the danger to human health has been significantly reduced or eliminated. Concurrently, this remediation technology may achieve substantial cost savings in comparison to the standard practice of soil drying and off site landfill deposition (Scheckel et al., 2009).

TH 104
Influence of humic-based sorbents on bioavailability of lead in soil M.A. Pukalchik1, V.A. Terekhova2, A.A. Izosimov2, O.S. Takanenko1, K.A. Hydralieva2 1Institute of Problem of Chemical Technology, Byasik, Kyrgyzstan 2Institute for the Protection of the Environment, Moscow, Russian Federation

Heavy metals are major pollutants in industrial soils. This study focuses on in-situ remediation of lead’s soil pollution in model experiment. Commercially available potassium humate BC-HumNa produced by Russian company from brown coal as well as magnetoreactive humic-based nanocomposite Fe3O4/HA formulated by coprecipitation method were tested in situ. Fe3O4 nanoparticles are spherical and their diameters vary from 7 to 16 nm. Modeling of lead pollution was conducted in pots on samples of the model soil (100 g soil; PB 320mg/kg). Humic sorbents was introduced to achieve a concentration of 0.0025 and 0.01% (by weight). Lead water-soluble fraction was measured using the spectrophotometry. Desorption after toxicity was valued in relation to standard toxicities of living organisms (representatives of higher plants, invertebrates crustacean, and bacteria). Results are presented and discussed, indicating that both humic-based sorbents modified lead bioavailability in soil. Apparently changing in the content of water-soluble lead, and the responses of test organisms on the impact of aqueous extracts of contaminated soil humate nanostructured substances showed a higher detoxifying potential and were more effective than sodium humate.

TH 105
Preliminary results of the zinc, copper and iron content in the feral oyster Striostrea margaritacea and the effects of them on the lysosome membranes Y. Slabber, R.G. Snyman, J. Odendaal Set.ac, 6th World Congress/SETAC Europe 22nd Annual Meeting 254 SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting

Preliminary results of the zinc, copper and iron content in the feral oyster Striostrea margaritacea and the effects of them on the lysosome membranes.

TH 106
Uranium fate in crayfish after different conditions of exposure. Focus on U accumulation and speciation in the cytosolic fraction of 2 target organs: gills and digestive gland. S. Frelot1, S. Moussac2, R. Gillet2, S. Simon2 1IRSN, St paul lez durane, France 2IPREM / LCBIE, Pau, France

Uranium analysis at the organ and sub-cellular levels is necessary to understand biodistribution, whereas uranium speciation in water column is quite well studied by modelling in correlation to its bioavailability, its speciation within living aquatic organism is poorly described despite a wide coordination chemistry and affinity for proteins. Uranium analysis at the organ and sub-cellular levels is necessary to understand biodistribution, whereas uranium speciation in water column is quite well studied by modelling in correlation to its bioavailability, its speciation within living aquatic organism is poorly described despite a wide coordination chemistry and affinity for proteins. Uranium analysis at the organ and sub-cellular levels is necessary to understand biodistribution.

TH 107
Selenium and Mercury immobilization using modified mackinawite as reactive layer in in-situ capping. M.R.M. Chaves, P.R.P. Gamberotti1, L.D. Delaune2, P.M. Buchele2 1University of Sao Paulo, Sao paulo, Brazil 2Indiana State University, St paul lez durance, France
precipitation and specific metal interaction with biomolecules during the fixation, transport and incorporation into a biological cell or tissue. So, such information is fundamental to better understand uranium bioavailability, accumulation after direct and trophic exposures and toxicity.

In the case of uranium the elucidation of its toxicity does not only go through the characterization of target-biomolecules involved in metabolic pathways but also through the determination of its uptake and distribution. This study deals with the uranium fate in two target organs of crayfish, i.e. gills and digestive gland, after different levels of direct exposure. As mainly considered as chemotoxic, focus was done on the soluble fraction of U in these organs. Thus, subcellular distribution of uranium was performed using ultracentrifugation followed by uranium specific ICP-MS analysis for quantitative determination. Results show that in crayfish, U accumulation in gills is higher than in digestive gland after direct exposure. Only 25 to 5% of U are in the soluble fraction of gill cells and can be considered as U speciation and the assessment of the chemotoxic fraction of this element. In digestive gland, the soluble fraction represents 60 to 30% of the uranium content.

The ICP MS chromatogram of DG cystos shows similar patterns for control samples and contaminated samples while intensity of 3 fractions at 4, 7 and 10 kDa seems to be enhanced with the contamination. Same patterns and trends were observed with the UV detection at 280 nm. To avoid ecotoxicological risk and to understand the biogeochemical cycling of mercury species in the environment. Since methyl mercury is the most toxic species, routine methods for the extraction and determination of methyl mercury and inorganic mercury in sediments and fish tissues using HPLC-ICPMS have extensively been used as chelating agents in many industrial and domestic applications for years.

To conclude, Hyphenated techniques were used to assess U rephosphorylation and U distribution within cytosolic proteins of cells from gills and digestive gland of crayfish after exposure. Three main protein peaks of interest have been identified and will further be analyzed to identify molecules complexed with U. These speciation studies correlated to biological effects are a key step to elucidate the chemotoxicity of this element.

**TH 107**

**Determination of Se species in fish tissues by HPLC-ICPMS**

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Se plays an important role in humans and animals as it is an essential component of a number of enzymes including glutathione peroxidase. The narrow margin between the beneficial and toxic levels of Se has important implications on human health. Due to its dual role, Se has been widely studied in the environment.

The release of Se from geological sources has been accelerated due to industrial and agricultural activities making it bioavailable to fish and other organisms in aquatic ecosystems, hence, Se has surfaced as an element of primary concern. Se is present in many chemical forms in the environment: from inorganic Se (IV) and Se (VI) to the organic amino acids such as selenocysteine and selenomethionine. The toxicity of Se is related to its chemical form with the inorganic forms being more toxic than the organic from in general. Many enzymes and other proteins require Se for their activity and the forms required by animals are the inorganic selenoaminoacids or Se containing proteins. Since, selenoaminoacids are mainly associated with proteins in living organisms, to release the Se species incorporated into proteins, the peptide bond needs to be broken in a way that releases the intact Se containing amino acids or their derivatives. Although, the measurement of total Se is essential to provide elemental concentrations, it is insufficient information if the biological actions of Se are dependent on the physical properties of its various chemical forms. Speciation information is therefore required to gain an understanding of its biogeochemical cycling and metabolism.

This project investigates the extraction and determination of Se species by HPLC-ICPMS after proteolytic digestion and the derivatisation of selenoaminoacids. Low efficiency of proteolytic extractions reported in the literature could be a consequence of incomplete digestion of tissue due to insufficient unfolding of proteins, hence the inaccessibility of the selenoamino acids. Methyl mercury is a neurotoxin that readily crosses biological membranes which accumulates to harmful concentrations in exposed organisms and biomagnifies in aquatic foodwebs to concentrations of toxicological concern.

Developing a sensitive, reliable and cost effective method to measure mercury species is important for monitoring mercury concentrations in biota and sediments in order to avoid ecotoxicological risk and to understand the biogeochemical cycling of mercury species in the environment. Since methyl mercury is the most toxic species, routine analysis of this species is increasingly in demand. This project describes a procedure for the extraction and determination of methyl mercury and inorganic mercury in sediments and fish muscle tissues using HPLC-ICP-MS after the extraction using 2-mercaptoethanol. A Perkin-Elmer 3 µm C8 (30 mm x 3 mm) HPLC column and mobile phase containing 0.5% v/v 2-mercaptoethanol and 5% v/v methanol (pH 5.5) at a flow rate of 1.5 ml min⁻¹, 250°C was used for the separation of mercury species. The developed extraction procedure of mercury species from the fish tissues was compared against an enzymatic extraction using Protease type XIV and similar results were obtained for both extraction procedures. The methyl mercury contamination in sediment reference material CRM C 380 and biological certified reference materials, NRC DORM-2 Dogfish muscle, NRCB Dole-3 Dogfish liver, NIST RM Albacore tuna and IRMM IEMP-20 tuna fish were in agreement with the certified values.

**TH 109**

**EDTA: a marker of anthropogenic pollution in the Suquia River basin**

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**Ambiental de eutrofización de la cuenca del río Suquia**, Córdoba, Argentina

To verify the pollution we also measured concentration of metals (Li, Be, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Sr, Mo, Ag, Cd, Ba, Ti, Pb and Bi), pH, conductivity and dissolved oxygen (DO) at eight monitoring stations with different pollution degree from the upper to the lower river basin. Sampling was carried out during 2011, considering both dry and wet seasons. EDTA was analyzed by HPLC coupled to ESI-HRMS (QTOF), while metals were assayed by ICP-MS. EDTA was analyzed at least 3 times, and each time a new EDTA was added. At each sampling site, race amount of EDTA varied among 3 locations. At high basin, race amount in the middle basin, while the highest values were observed at the lower basin. So far, it is possible to differentiate 3 areas in accordance with anthropogenic inputs: a quasi pristine area in the upper basin, a medium polluted zone surrounded by small cities in the medium basin, and a high impacted area, downstream the sewage discharge of the main city (Córdoba). Some metals showed a high level of conductivity, DO and other metals (Li, K, Ca, Mg, Ga, Sr, Ba) showed a pattern common with EDTA concentration, with increased values for anthropogenic influence rise. Additionally, concentrations of Al, Mn, Fe, Cu and Zn increased progressively after Córdoba sewage discharge. Concentrations were generally higher during the dry season. Our current results demonstrate that EDTA can be used as an efficient marker of anthropogenic pollution, in addition to metals and other water quality parameters.

**TH 110**

**Speciation and bioavailability of arsenic and heavy metals in contaminated stream sediments**

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**The application of the Selective Chemical Extraction technique on the sediments of the stream that drains the “Vale das Gatas” Mine area, allowed us to notice that:** 1) the most part of the contents of Ca, W, Sn, Ni and Mn in the residual fraction, is mainly constituted by silicate minerals, non bioavailable; 2) part of the metals (Fe, Mn, Cu, Zn, Cd and Pb) are bioavailable in all sampling sites in large percentages; 3) all the researched metals are also potentially bioavailable, that is, they occur in organic, reducible and carbonate phases, susceptible of being set free depending on some changes of water stream physical-chemical conditions. Cu, Zn, As, Cd, and Pb are the most remarkable examples. The characteristics of the main contamination focus, also appear as an important aspect, conditioning the support phases of the researched chemical elements. Thus, in station where metal to stream contamination is essentially owed to mining activity (AMD), a great part of the elements appear preferentially associated to exchangeable and moderately reducible fractions. That is a result of the affinity of these elements in being adsorbate and its easiness of coprecipitating with Fe oxides; while in stations where the contamination is owed to domestic sewage discharge, some metals only are associated to exchangeable fractions. So, such information is important for a successful environmental management, and for the comprehension of a potential contamination scenario.
One response to plants to exposure to arsenic is the production of Glutathione (GSH), although its part in the detoxification mechanism is still unclear. Due to the strong affinity of arsenic for sulphur, glutathione (GSH) and phytochelatins (PC) tend to form complexes with As(III) and to a lesser extent, As(V). However, the identification and quantification of these complexes has proven to be elusive due to the instability of the metalloid complexes and it represents a challenge for modern speciation techniques. Arsenic speciation instrumentation has received much recent attention and reliable speciation has been achieved. In contrast, relatively little progress has been made to improve sample storage and extraction protocols. In particular, extraction of As in the microalgae Chlorella vulgaris remains poor impeding As-GSH complexes identification in these cells.

The aim of this investigation was to determine the presence of As-GSH complexes in Chlorella vulgaris after exposure to arsenic and contribute to elucidate the mechanisms of arsenic detoxification used by microalgae. For this reason synthetic As-GSH complexes were prepared. Chlorella vulgaris cells were exposed to As(III) and As(V), concentrations ranging from 0-200μM.

The quantification and determination were performed by extraction with HPLC-ICPMS and HPLC-ESI-MS. The same chromatographic conditions were used for both instruments. Quantification by ICPMS does not ensure unequivocal identification of molecules but allows the determination at low detection limits and is not significantly affected by matrix effects. The use of ESI-MS/MS allows both low concentration quantification and unequivocal identification of molecules of interest. In order to understand the mechanism of detoxification employed by living cells exposed to inorganic arsenic, reliable methods for extraction, quantification and detection must be developed. In this study, sample preparation and extraction procedure appropriate for the nature of the analyte has been applied to minimise oxidation and preparation time. Trace analyte quantification with low limits of detection and low matrix effects by ICP-MS and correct identification of the As-GSH with ESI-MS was achieved in Chlorella vulgaris cells exposed to inorganic arsenic.

**TH 112**

**LC-ICP-MS speciation of selenium compounds in Se-enriched green algae**

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Selenium is an essential element and has been known to be a necessary component of the human diet. It is important e.g. for protection of cells against effects of free radicals and for the normal function of immune system. Selenium is also toxic and its toxicity and bioavailability is related to concrete selenium compound. Usually organic selenium species (Se-amino acids) exhibit higher bioavailability and lower toxicity than inorganic selenium compounds. Se-enriched biomass of green algae Scenedesmus and Chlorella might become a source of non-protein bound selenium amino acids and inorganic ions. Supported by CETOCOEN (CZ.1.05/2.1.00/01.0001) project granted by the EU and administered by the Ministry of Education, Youth and Sports of the Czech Republic. The authors also are grateful for financial support of MSMT (MSM0021622412).

**TH 113**

**Speciation of arsenic and selenium in surface water**

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With a focus on the analysis of various international River basin programs arsenic pollution is monitored in Dutch surface waters, among other matrices. Selenium is monitored on a national basis. To determine the impact on the aquatic environment of the amounts present (national) environmental quality standards are used. Current research into the speciation of both elements should lead to a better understanding of the extent of pollution, because of the various species differs extensively. This approach is expected to be incorporated into future legislation frameworks.

**TH 114**

**Ion chromatography with XSERIES 2 for metal speciation applications**

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1. Introduction

Here, the coupling of the Dionex ICS-5000 ion chromatography system to XSERIES 2 is described. This combination provides a single powerful, flexible solution for both ion chromatography and ion exchange chromatography applications as well traditional HPLC methods. The combined system is ideally suited for trace metal speciation as it provides the system with a wide selection of chromatographic columns applicable, appropriate solutions for all types of trace metal speciation studies can be found.

2. Materials and methods

In this study, speciation analysis of different trace metals was carried out using hyphenation of the Dionex ICS-5000 chromatography system coupled to XSERIES 2. Different analytical columns were used including anion exchange chromatography and reversed phase chromatography.

3. Results and discussion

Different application examples of the system will be shown, including from ion chromatography (e.g. Cr speciation), ion exchange chromatography (speciation of different As compounds), and also reversed phase chromatography for the separation and quantification of organotin species. Particular emphasis will made on the use of smaller internal diameter columns that significantly lower the consumption of mobile phase (and therefore sample) without compromising separation efficiency.

4. Conclusions

The results show the possibilities offered by the hyphenation of the Dionex ICS-5000 LC system to XSERIES 2 for trace metal speciation analysis. Due to the high flexibility offered by the system, different applications could be successfully run on the same instrument and showed high separation efficiency together with reduced sample and mobile phase consumption.

**TH 115**

**Speciation of Bromine in water samples using HPLC-ICP-MS**

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Foods and agricultural products contain various concentrations of nutrients, which can range from trace amounts to percentage levels. However, whilst most of their nutritional value is needed for maintaining good health, the benefits of the desired mineral content of such products can be compromised by concentrations of elements deemed to be toxic to humans and/or animals.

ICP-MS is a flexible tool for minor, trace and elemental analysis. In combination with separation techniques, ICP-MS allows the determination of the chemical form of elements in a single sample and thereby a differentiation between essential nutrient and toxic to human or animals. For elements such as As, Cr, Se and Br it is well known that it depends on the oxidation state or the present compound if an element is harmless or toxic to humans. Bromate e.g. is formed by the ozonation of water in purification processes. Bromate is a potential carcinogen whereas bromide is essentially non-toxic. Therefore monitoring of the total bromine concentration in water will not indicate the risk for human health. The coupling of a chromatography technique to an ICP-MS provides a powerful and very sensitive tool to separate bromine and bromate. The presentation will imply the required sample preparation steps, flexibility and sensitivity for the determination of different bromine species in water samples using the Bruker aerazon90 ICP-MS. The capability of this method for this kind of analysis is for this reason of great interest.

**TH 116**

**New approaches for the traceable determination of PBDEs (in coastal water samples) according to the Water Framework Directive (WFD)**

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Polychlorinated dibenyl ethers (PBDEs) have been widely used as flame retardants in polymeric materials. Owing to the adverse effects linked to the exposure to these compounds, the EU has included PBDEs (congeners 28, 47, 99, 100 153 and 154) in the list of priority substances that need to be measured in all surface waters covered by the WFD. Environmental Quality Standards (EQS) exist for the maximum allowable concentrations, have been defined at very low levels for PBDEs (2PBDEs ≤ 0.5 ng/L). Analytical methods intended for their monitoring must meet certain minimum performance criteria in terms of uncertainty (≤ 50% at EQS, 95% confidence) and limits of quantification (LOQ ≤ 30% of EQS) for reliable measurements of PBDEs in complex environmental water matrix at EQS level. The overall low concentrations of the target analytes in water together with the challenging requirements defined by the WFD necessitates the development of suitable methodologies combining sensitive detection approaches with accurate and precise analytical techniques.

In this study, a solid-phase extraction technique (SPE-ICP-MS) or electron capture negative ionization (ECNI) mass spectrometry provide interesting possibilities as it offers high sensitivity for the detection of low concentration such as bromine, especially when using gas chromatography (GC) as sample introduction technique. Furthermore, ICP-MS has been widely used in combination with Isotope Dilution Mass Spectrometry (IDMS) for the accurate and precise determination of other priority pollutants. In this work, some preliminary results on the determination of PBDEs in water by elemental IDMS are shown. Ultrapure water samples fortified at the EQS-level were spiket with analytically pure PBDEs, dissolved in liquid-liquid extracted residuals. The requirements of the WFD in river water samples but LOQs still need to be further improved. Instrumental settings of the GC-ICP-MS coupling were optimised for the highest bromine sensitivity for its future use in IDMS as an attempt to meet the WFD requirements.

This work has been performed within the scope of an EMRP Researcher Grant for the development of a traceable measurement approach for monitoring PBDEs in coastal water, awarded in accordance with the EURAMET process to complement the JRP “Traceable measurements for monitoring critical pollutants under the European Water
Improved extraction, preservation and analysis of hexavalent Chromium from soil extracts and water

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The emergence and the dissemination of antibiotic resistance constitute a global threat for public health and an emergent environmental problem. Rivers and streams constitute a reactor for evolution of new resistance because bacteria from different sources such as urban, industrial waste water, or farms, are mixed with indigenous species. Heavy metal exchange between allochthonous and indigenous bacteria appear to be promoted by inorganic and organic pollution; however current knowledge is largely incomplete. As a case study, we investigated the antibiotic resistance features of the Gram-negative populations of the Bug River (Ukraine) and its main tributary, the Poltva and downstream in the Bug, four β-lactams resistant E. coli harbouring blaCTX-M-14 and one blaCTX-M-1 were found. Additionally, the acquired fluoroquinolones resistance gene qnrS was detected in three isolates. Class 1 integrons were present in 16 of the resistant E. coli. For two isolates, the insertion sequence ISEcp1, involved in promoting antibiotic resistance has been detected, leading to the conclusion that these contaminants are key for the development of antibiotic resistance in the environment.
the mobilisation of blaCTX-M genes, was found ahead of blaCTX-M-14 gene. RAPD analysis indicated a relatedness between four Escherichia coli harbouring blaCTX-M-14 of which two were isolated from different sampling sites. The MLST profile indicated that the E. coli harbouring blaCTX-M-14 belonged to the ST131 and the one harbouring blaCTX-M-1 belonged to ST156. This study, integrating environmental and clinical approaches, is the first report of antibiotic resistance genes isolated from water bodies in the Baltic Sea. Furthermore, horizontal gene transfer is an important case study demonstrating how organic pollution of rivers may represent a critical risk: the water acts like an infectious source and reservoir of antibiotic resistance.

**TH 125**

Distribution of novel beta-lactam resistance genes of ampC family among environmental aquatic bacteria

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In addition to medical setting, antibiotic resistance is widespread in environmental aquatic bacteria. One factor contributing to rapid spread of antibiotic resistance is horizontal gene transfer. For detecting the horizontal transfer, phylogeny of the resistance genes has to be compared to the phylogeny of the hosts. In the current work we focused on the isolation of novel beta-lactam resistant Acinetobacter baumannii strains from the run off river the bacteria were isolated from a lake and its run off river. The bacteria were isolated on antibiotic (including ampicillin) containing selective media. The isolation collection was obtained from samples collected in years 2005 to 2008. The lake is potentially exposed to several antibiotics from use in farming and diffuse countryside settlements while there is a larger town nearby. The bacteria isolated were tested for resistance against various beta-lactam antibiotics and were very resistant. We identified and typed based on the analysis of the rpoB, gyrB and recA partial gene sequences. Antibiotic resistant bacteria are persistent in the sediment below aquaculture farms even in the absence of selection pressure. Here, HPLC analyses were conducted to monitor the selection pressure in the aquaculture farm sediment.

Betaproteobacterium. The total number of FOX containing isolates was 44. The most prominent group of FOX resistance genes was genus Aeromonas (n = 26), most of them the fish pathogen Aeromonas salmonida. On the other hand, not all isolated Aeromonas bacteria (n ~ 60) had FOX genes. In addition, the FOX gene was abundant among Pseudomonas. According to the sequences the FOX genes found clustered to form a novel subclass, related to the plasmid borne FOX (FOX-3, -4, -8 and -9) sequences found in the Enterobacteria Escherichia coli and Klebsiella spp. We also found that the phylogeny of FOX genes does not coincide with the phylogeny of the host bacteria. In conclusion, we have identified a new subclass of ampC genes where transfer between different bacterial groups has been relatively frequent.

**TH 126**

Emergence of antibiotic resistant bacteria in Baltic Sea aquaculture

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The emergence and dissemination of antibiotic-resistant bacteria (ARB) is a major global health concern. Antibiotic resistance is a threat to public health due to the rapid spread of antibiotic-resistant bacteria (ARB) within and between species and environments. The Baltic Sea is an important aquaculture area in Europe and receives effluents from hospitals, sewage treatment plants (STP) and urban areas. The aim of the current work was to assess the impact of hospitals effluents on the STP and from the RAPD pattern of the ESBL producing isolates.

**TH 127**

Diversity and antibiotic resistance of Acinetobacter spp. in water from the source to the tap

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Significant amounts of antibiotic resistant Acinetobacter spp. are released daily into the environment. The prevalence and molecular diversity of resistance genes may be an indicator of water quality, useful to assess the risk of antibiotic resistance to public health. Production of extended-spectrum beta-lactamases (ESBLs) is one of the characteristics of Acinetobacter spp. that Gram-negative bacteria have developed as the way to survive against the increasing usage of broad-spectrum antibiotics such as cefotaxime (CTX). The association of ESBL genes with genetic elements that potentiate their capture and expression have contributed to their highly efficient mobilization and expansion, being reported frequently worldwide and thus becoming a serious clinical threat. The aim of this study was to compare the prevalence and diversity of CTX-M resistant (CTX-M) bacteria within polluted (P) and unpolluted streams. The twelve rivers were selected in Portugal (6) and the Ukraine (6) for their high organic content and phylogenetic diversity. In the current work we focused on the horizontal gene transfer. Mobile integrons (MI) are genetic elements that acquire, exchange and express antibiotic-resistance genes embedded within gene cassettes (GC). Class 1 MI are the most frequent with more than 130 GC conferring resistance to almost all known antibiotics. The aim of this study was to assess the class 1 MI relative abundance and their GC composition in an hospital and an urban effluent. The relative abundance of class 1 MI in hospital effluents was 9 and 7 fold higher than in urban effluents, suggesting that hospital effluents are an important source of beta-lactams resistant bacteria. Moreover, MI harboured genes involved in resistance to aminoglycoside, beta-lactams, trimethoprim, chloramphenicol, erythromycin, and quaternary ammonium compounds. The Choab abundance based Jaccard similarity index was used to compare the shared GC pool between samples. In conclusion, results showed clear differences between P and UP lotic ecosystems, concerning prevalence, phylogenetic diversity and antimicrobial susceptibility profiles of CTX-M and FOX resistance genes.

**TH 128**

Resistance to broad-spectrum antibiotics in aquatic systems: do anthropogenic activities affect the dissemination?

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University of Aveiro, Aveiro, Portugal

Mobile integrons (MI) are genetic elements that acquire, exchange and express antibiotic-resistance genes embedded within gene cassettes (GC). Class 1 MI are the most frequent with more than 130 GC conferring resistance to almost all known antibiotics. The aim of this study was to assess the class 1 MI relative abundance and their GC composition in an hospital and an urban effluent. The relative abundance of class 1 MI in hospital effluents was 9 and 7 fold higher than in urban effluents, suggesting that hospital effluents are an important source of beta-lactams resistant bacteria. Moreover, MI harboured genes involved in resistance to aminoglycoside, beta-lactams, trimethoprim, chloramphenicol, erythromycin, and quaternary ammonium compounds. The Choab abundance based Jaccard similarity index was used to compare the shared GC pool between samples. In conclusion, results showed clear differences between P and UP lotic ecosystems, concerning prevalence, phylogenetic diversity and antimicrobial susceptibility profiles of CTX-M and FOX resistance genes. This validates the hypothesis that anthropogenic activities modulate the environmental resistance gene pool and are promoting antibiotic resistance dissemination. Also, it suggests that CTX-M-like genes may constitute pollution indicators.

**TH 129**

Abundance of class 1 integrons and diversity of their associated gene cassettes in hospital and urban effluents

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University of Limoges, Limoges cedex, France

Waste waters are considered to be sources for antibiotic resistance dissemination by horizontal gene transfer. Mobile integrons (MI) are genetic elements that acquire, exchange and express antibiotic-resistance genes embedded within gene cassettes (GC). Class 1 MI are the most frequent with more than 130 GC conferring resistance to almost all known antibiotics. The aim of this study was to assess the class 1 MI relative abundance and their GC composition in an hospital and an urban effluent. The relative abundance of class 1 MI in hospital effluents was 9 and 7 fold higher than in urban effluents, suggesting that hospital effluents are an important source of beta-lactams resistant bacteria. Moreover, MI harboured genes involved in resistance to aminoglycoside, beta-lactams, trimethoprim, chloramphenicol, erythromycin, and quaternary ammonium compounds. The Choab abundance based Jaccard similarity index was used to compare the shared GC pool between samples. In conclusion, results showed clear differences between P and UP lotic ecosystems, concerning prevalence, phylogenetic diversity and antimicrobial susceptibility profiles of CTX-M and FOX resistance genes. This validates the hypothesis that anthropogenic activities modulate the environmental resistance gene pool and are promoting antibiotic resistance dissemination. Also, it suggests that CTX-M-like genes may constitute pollution indicators.

**TH 130**

Occurrence of antimicrobial resistance among environmental bacterial communities of fish farms and pond sediments

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Antibiotics are the most important therapeutic agents used in control of bacterial infectious diseases. The use of a wide variety of antibiotics in human medicine and animal husbandry, including aquaculture, has led to the emergence of antibiotic resistant pathogens. In the present study bacteria from water and sediments related to aquaculture were isolated from lakes in the north of Portugal (135 isolated) and Northern Italy (80 isolated). The bacterial isolates were screened for the presence of prevalent antibiotic resistance genes by a disc diffusion assay, followed by Southern hybridization and further confirmation by PCR and gene sequencing. The marine aquaculture industry in Japan uses antibiotics extensively while the marine aquaculture industry in Norway uses low levels of antibiotic treatment. Freshwater aquaculture in Portugal and Italy are at risk of contamination of antibiotics but however, in higher in fresh water isolates than in marine isolates. The prominent resistance genes found in the bacterial isolates studied were Tet-A, Tet-G and Tet E operons (7.6 % of isolates), sul1 and sul2 (23.5 % of isolates), 

Furthermore, we want to investigate the correlation between increased level of resistance and measures of human impact on the lakes, e.g. degree of eutrophication, presence of intensive agriculture or waste water treatment plants in the watershed, etc. The aim of the presented study is to evaluate the prevalence of antibiotic resistance genes in different lakes in Switzerland, a country with comparatively low antibiotic use. Antibiotic resistance in clinical environments is a major concern in human and veterinary settings as it interferes with infectious disease treatment and raises health costs. Moreover, antibiotics and antibiotic resistant bacteria are currently discussed as a new class of environmental pollutants as they are released in high amounts from treated animals and waste water facilities into natural environments, including natural waters, where their fate and impact are yet unknown. Elevated discharge has likely lead to an increase in the natural resistance background level of the exposed environments, such as surface waters and soils.

The dataset will provide a basis to determine background levels of the natural antibiotic resistance versus antibiotics resistance pollution and to propose suitable indicators.

**TH 113 Increased antibiotic resistance levels in Swiss lakes are related to human activities**

C. Z. S. Sigdel, H. W. Brüggmann, Eawag, Kastanienbaum, Switzerland

The presence of antibiotic-resistant bacteria in clinical environments is a major concern in human and veterinary settings as it interferes with infectious disease treatment and raises health costs. Moreover, antibiotics and antibiotic resistant bacteria are currently discussed as a new class of environmental pollutants as they are released in high amounts from treated animals and waste water facilities into natural environments, including natural waters, where their fate and impact are yet unknown. Elevated discharge has likely lead to an increase in the natural resistance background level of the exposed environments, such as surface waters and soils.

The aim of the present study is to evaluate the prevalence of antibiotic resistance genes in different lakes in Switzerland, a country with comparatively low antibiotic use. Furthermore, we want to investigate the correlation between increased level of resistance and measures of human impact on the lakes, e.g. degree of eutrophication, presence of intensive agriculture or waste water treatment plants in the watershed, etc.

**TH 132 Differential gene expression and biofilm formation of multiple antibiotic-resistant Pseudomonas aeruginosa in municipal wastewater**

A. R. Varela, J. Hübener, E. Grohmann

University Medical Center Freiburg, Freiburg, Germany

Wastewater (WW) reuse for irrigation is widely practiced in agriculture to alleviate water shortages. WW contains a huge amount of potentially harmful compounds, such as pharmaceuticals and microbial pathogens. We investigated the risks of WW irrigation in the Mézquital Valley (60 km north of Mexico City) for environment and human health. The Mézquital Valley is the world’s largest irrigation field. WW from Mexico City is reused for crop irrigation, which poses risks for farmers and the consumers of the agricultural products. We followed the pathway of the WW from Mexico City to the Mézquital, starting with the WW effluent of two hospitals in Mexico City. In addition we took samples from the two major WW channels which transport the WW out of the city to the Mézquital. The influent and effluent of a dam in the Mézquital, and the reclaimed water after passing through the soil were sampled subsequently. Sampling was done in March and September 2011 to study differences between dry and rainy seasons. We selected two frequently used antibiotics, sulfamethoxazole and ciprofloxacin, and five antibiotic resistance genes (sul1, sul2, qnrA, qnrB, qnrS) that mediate resistance against these antibiotics. Furthermore, the blaSHV5 gene that is frequently found in bacterial isolates from patients in Mexican hospitals was selected. This gene encodes for Extended Spectrum Beta-Lactamases (ESBLs). Furthermore, we determined the concentration of the faecal indicator Enterococcus spp. and the total bacterial load. A total of 168 RNA genes was extracted from the WW samples and the prevalence of the antibiotic resistance genes was measured using SYBR Green PCR, and the total bacterial load was determined using qPCR. Conclusive that the prevalence of the antibiotic resistance genes was, however, much lower than expected. The predominating antibiotic resistance genes were identified and the resistance genes were present in low copy numbers. The blaNDM-1 was not found. All isolates were also negative for plasmid mediated qnrA, qnrB and qnrS genes.

**TH 133 Occurrence of antibiotic resistance genes in wastewater used for irrigation in the Mézquital Valley**

M. B. Roszecat, J. Hübener, E. Grohmann

University Medical Center Freiburg, Freiburg, Germany

The presence of antibiotic-resistant bacteria in clinical environments is a major concern in human and veterinary settings as it interferes with infectious disease treatment and raises health costs. Moreover, antibiotics and antibiotic resistant bacteria are currently discussed as a new class of environmental pollutants as they are released in high amounts from treated animals and waste water facilities into natural environments, including natural waters, where their fate and impact are yet unknown. Elevated discharge has likely lead to an increase in the natural resistance background level of the exposed environments, such as surface waters and soils.

The aim of the present study is to evaluate the prevalence of antibiotic resistance genes in different lakes in Switzerland, a country with comparatively low antibiotic use. Furthermore, we want to investigate the correlation between increased level of resistance and measures of human impact on the lakes, e.g. degree of eutrophication, presence of intensive agriculture or waste water treatment plants in the watershed, etc.

**TH 114 Development of treatment methods for the degradation of antimicrobial compounds present in wastewaters**

A. C. Reis, C. M. Manaia, O. C. Nunes

Escola Superior de Biotecnologia - Universidade Católica Portuguesa, Portugal

The use of antibiotics in the treatment of infectious diseases and disinfection leading to the accumulation of these contaminants in the environment and with the sewage from the hospitals, and other serious environmental concerns. The transportation of these compounds by surface and ground waters can contaminate different areas. The degradation of these contaminants is necessary to environmentally friendly. This study aims at developing low cost effective treatment systems to remove beta-lactams from wastewaters, by using microorganisms able to degrade these antibiotics. Biodegraders thriving in wastewater treatment plants have been enriched using amoxicillin supplemented culture media. Mixed cultures able to transform approximately 100 % of 30 mg L⁻¹ amoxicillin to non-active degradation products were obtained. The members of these cultures were identified and their antibiotic resistance phenotype was determined. The genetic determinants responsible for their degrading activity were also characterized. The diversity of bacteria and of genes involved in these processes will be also discussed.

**TH 135 From the hospital effluent to the municipal wastewater treatment plant: bacterial communities and antibiotic resistance**

A. Varela, C. M. Manaia, O. C. Nunes

Escola Superior de Biotecnologia - Universidade Católica Portuguesa, Portugal

The use of antibiotics in the treatment of infectious diseases and disinfection leading to the accumulation of these contaminants in the environment and with the sewage from the hospitals, and other serious environmental concerns. The transportation of these compounds by surface and ground waters can contaminate different areas. The degradation of these contaminants is necessary to environmentally friendly. This study aims at developing low cost effective treatment systems to remove beta-lactams from wastewaters, by using microorganisms able to degrade these antibiotics. Biodegraders thriving in wastewater treatment plants have been enriched using amoxicillin supplemented culture media. Mixed cultures able to transform approximately 100 % of 30 mg L⁻¹ amoxicillin to non-active degradation products were obtained. The members of these cultures were identified and their antibiotic resistance phenotype was determined. The genetic determinants responsible for their degrading activity were also characterized. The diversity of bacteria and of genes involved in these processes will be also discussed.
Furthermore, the influence of the hospital effluents on the bacterial communities of the WWTP was investigated using 16S rRNA PCR DGGE. Cell counts and amoxicillin resistance rates were similar between the hospital discharge and the point of inflow to the WWTP. In contrast, ciprofloxacin resistance was detected to be about three times higher in the final effluent than in the raw municipal wastewater. The hospital discharge seems to have a significant impact on the levels of ciprofloxacin resistance among the bacterial communities of the WWTP, with approximately 100-1000 times for both total and AR bacteria. However, the treatment did not cause significant variations on the rates of amoxicillin and ciprofloxacin resistance. This study confirms hospital discharge as a significant source of AR into the environment. The variation in bacterial resistance in the hospital effluent and in the WWTP raw and treated effluent is analyzed.

TH 136
Phylogenetic diversity of quinolone resistant Escherichia coli isolated from wastewaters
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Antibiotic resistance dissemination has become more important in the last decades. The species Escherichia coli comprises different subgroups which may have different roles in antimicrobial resistance dissemination. In previous studies, wastewater treatment was observed to promote an increase in quinolone resistance, presumably due to the different selectivity of distinct genotyping groups. Figueira et al., 2011, have shown that antibiotic resistance genes (ARGs) of quinolone-resistance genes and the phylogenetic group of the bacteria. Severity Escherichia coli strains isolated from raw and treated municipal wastewaters were characterized based on a multi locus sequence typing (MLST) approach. The presence of quinolone resistance determinants was assessed based on the detection of mutations in the gyrA and parC genes and on the presence of the genes

TH 137
Antibiotic resistance and bacterial communities in a wastewater treatment plant
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Although it is still difficult to establish clear cause-effect relationships, it is widely accepted that chemical pollution in the environment can influence the spread of antibiotic resistance among bacterial communities. Domestic wastewater is a complex mixture of environmental and human commensal bacteria, which come in contact with a considerable amount of antimicrobial agents and other substances with unknown effects on microbial communities and on bacterial metabolism.
In this work we studied the bacterial communities, based on DGGE patterns and used multivariate analysis to infer about the influence of some environmental parameters (antimicrobial concentrations, percentages of antibiotic resistance among culturable bacteria, and climatic and plant operation conditions).
This study was designed to identify the factors observed to influence or be interdependent of either the bacterial communities and/or the antibiotic resistance rates. A total of 14 composite samples of raw and treated wastewater were collected from an activated sludge wastewater treatment plant over a period of about six months. Total DNA was extracted from samples and used for the DGGE analysis. Antibiotic and heavy metals were determined in the inflow and in the treated effluent. The culturable antibiotic resistant rates were documented on Novo & Manaia, 2010 study. Some of the most relevant DGGE bands were selected for further identification. Based on the obtained DGGE profiles, bacterial communities formed coherent groups according to the time of the year. Among the bacterial taxa varying over these periods were, for example, Arcobacter, Streptobacillus, Firmicutes and Clostridiales in raw wastewater and Sulfitomomas, Bacteroidetes, Leadibeterea, Grammaproteobacteria, Arcobacter and Streptobacillus in treated wastewater. After a Phylogenic Analysis, it was made possible to assess the influence of some factors and to find significant relationships between DGGE bands, antibiotic resistance rates and antimicrobials concentrations.
However, the direct influence of a specific antibiotic resistant bacterial group or antimicrobial could not be identified in the community structure.
The results suggest that the wastewater communities are affected by the presence of antimicrobial agents and that these substances affect the bacterial metabolism which may be related to antibiotic resistance dissemination.

TH 138
Quantification of resistance genes and presence of resistant bacteria in hospital effluents from wastewater treatment plants
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Antibiotic resistance is increasing rapidly among bacteria as antibiotics are used in increasing quantities in treating humans and animals. Due to bacterial multiresistance to different antibiotics, we are heading back to pre-antibiotic era where there is no treatment for common bacterial infections. One major concern is the microbial resistance for the so-called last resort antibiotics, such as vancomycin, carbapenems and quinolones.
In wastewater treatment plants antibiotics, other pharmaceuticals, heavy metals and xenobiotics mix up with bacteria from human, animal and environmental origin. Wastewater treatment plants are known reservoirs for various resistance determinants and serve as hotspots for horizontal gene transfer. Resistance genes for different antibiotics have been transferred between mobile genetic elements that are able to horizontally transfer between different bacteria. Based bacterial populations and biotools in the sludge promote horizontal gene transfer and different chemicals can pose a selection pressure promoting the survival of the resistant bacteria. Horizontal transfer of certain mobile genetic elements, such as Tn916, has also been shown to be inducible by antibiotics. The resistant bacteria from the wastewater treatment plants may eventually end up in the environment either in effluents or in the treated water.
In this study we quantified resistance genes for the last resort antibiotics, such as carbapenems, 3rd & 4th generation cephalosporins, quinolones and vancomycin, from different compartments of the wastewater treatment plant using quantitative PCR. Moreover resistant bacteria were cultured on ciprofloxacin and meropenem containing media and the resistance pheno- and genotypes were characterised.
Our results show that many resistance determinants and heavy metal bacteria can be found from the wastewater treatment plants. Resistance genes and resistant bacteria are even more found from the effluents via which they are released to the environment.

TH 139
Vancomycin and ciprofloxacin resistance in enterococci from a Hospital effluent and in the receiving Municipal Wastewater Treatment Plant
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The increasing incidence of resistance to a wide range of antibiotic agents in human commensal and pathogenic bacteria has become a major concern over the last decades. Enterococci are the main agents as vancomycin-resistant enterococci and quinolone-resistant enterococci.
In raw wastewater and in the treated effluent. The culturable antibiotic resistant rates were documented on Novo & Manaia, 2010 study. Some of the most relevant DGGE bands were selected for further identification. Based on the obtained DGGE profiles, bacterial communities formed coherent groups according to the time of the year. Among the bacterial taxa varying over these periods were, for example, Arcobacter, Streptobacillus, Firmicutes and Clostridiales in raw wastewater and Sulfitomomas, Bacteroidetes, Leadibeterea, Grammaproteobacteria, Arcobacter and Streptobacillus in treated wastewater. After a Phylogenic Analysis, it was made possible to assess the influence of some factors and to find significant relationships between DGGE bands, antibiotic resistance rates and antimicrobials concentrations. However, the direct influence of a specific antibiotic resistant bacterial group or antimicrobial could not be identified in the community structure.
The results suggest that the wastewater communities are affected by the presence of antimicrobial agents and that these substances affect the bacterial metabolism which may be related to antibiotic resistance dissemination.

TH 140
Occurrence of beta-lactamases, namelyGES-5 carbapenemase, among Gram-negative isolates from wastewater samples in Northern Portugal
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Background: Antimicrobial resistant pathogens are profoundly relevant to human health and many were the studies that focused on their spread. However, natural and human associated environmental reservoirs of antibiotic resistance (AR) are yet poorly understood. The main goal of this study was to evaluate some AR mechanisms in Gram-negative bacteria isolates from surface and raw and treated wastewater environments.
Methods: Water samples were collected from different aquatic environments within an urban water cycle in the region of Northern Portugal (1, 2). Screening of antimicrobial susceptibility of 48 Gram-negative isolates (20 Escherichia coli, 8 Citrobacter spp., 7 Klebsiella spp., 6 K. oxytoca spp., 2 Enterobacter spp., 1 Haffnia alvei, 1 Pantoea agglomerans, 1 Pseudomonas lutetiae, 1 Rickettsia ornithomiyctis, 1 Serratia spp.) was performed by disk diffusion method. The search for antibiotic resistant genes was based on the PCR identification of the susceptible and resistant loci. Some conserved sequences were used to screen and identify bla and plasmid-mediated quinolone resistance (PMQR) genes.
All isolates were also screened for the presence of class 1 integrons.
Results: Overall, 13 isolates were multidrug resistant, suggesting a great diversity of AR mechanisms. Two isolates showed non-susceptibility to carbapenems, one of the last resorts on the antimicrobial therapy. Their phenotype and molecular characterisation revealed the expression of a chromosomal metallo-beta-lactamase in P. beta lactamase with the presence of a GES-5 encoding gene in a Klebsiella pneumoniae isolate. Furthermore, a variety of beta-lactamase encoding genes were observed, specifically 12 bla groups, with
distinct promoters, 4 blaOXA-23 (2 blaOXA-48, and 2 blaOXA-51) and also different chromosomal AmpC beta-lactamases, namely CMY-65. Class 1 integrons were detected among 60 of TEM-1-producing isolates. Together, these beta-lactamases explain the level of beta-lactam resistance. None PMQR genes were detected.

Conclusion: In conclusion, this study provides the first description of a class A carbapenemase in an environmental setting in Portugal, in addition to several other beta-lactam resistance mechanisms. The study highlights the need of surveillance of these AR mechanisms in environmental backgrounds because it represents a liable reservoir of potential pathogenic resistant bacteria.


TH 141

Pseudomonas spp. in drinking waters - diversity and antibiotic resistance
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Pseudomonas are common inhabitants of aquatic environments, including drinking water. The problems associated with multi-resistance in clinical isolates of P. aeruginosa are well known, but a lack exists in the study of the presence of antibiotic resistance phenotypes in other species and in environmental isolates of this genus. This study aimed to evaluate antibiotic resistance in drinking water. Isolates from a drinking water treatment plant, household taps, cup fillers of dental chairs, a drinking water biofilm and bottled mineral waters were examined. Based on the analysis of the 16S rRNA gene sequence, it was observed that the isolates belonged to 20 species. Taxonomic diversity was further inferred based on the analysis of six housekeeping genes (16S rRNA, rpoD, rpoB, gyrB, recA, rrs) and FPGs. All isolates showed diffusion test results for beta-lactam production and sensitivity test results for the prevalence of antibiotic resistance was in, general, low, but related with the species or even with the ST. Resistance to ticarcillin, fosfomycin and ciprofloxacin were the most prevalent. Ceftazidime resistance was very rare, but was present in all the isolates identified as P. simiae. Resistance to nalidixic acid was observed in P. chlororaphis, P. nitroreducens, and P. aeruginosa recovered from tap water but not from biofilm. Intrinscic resistance was observed to cephalothin, and no resistance phenotypes were observed to ciprofloxacin, tetracycline, pipercillin, imipenem, meropenem and colistin.

This study shows that the Pseudomonas spp. observed in the taps may not have origin on the drinking water treatment plant. Antibiotic resistance was observed to be mainly species, rather than site-related. In general, Pseudomonas spp. are confirmed as potential vectors of antibiotic resistance in drinking waters, with the selection of some strains representing an important role of resistance spreading.

TH 142

Bottled mineral water as a potential source of antibiotic resistant bacteria
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The hypothesis that bottled mineral water may represent a supplier of antibiotic resistant bacteria to humans was tested. The antibiotic resistance phenotypes of the cultivable bacteria present in nine batches of two Portuguese and one French brands of commercially available mineral waters were examined. Among the 238 isolates recovered on R2A and PRA and on these culture media supplemented with amoxicillin or ciprofloxacin, most were identified (based on 16S rDNA gene sequence analyses) as Proteobacteria of the divisions Beta, Gamma and Alpha. Also, present, were members of the phyla Firmicutes, Actinobacteria and Bacteroides. Bacteria resistant to more than three distinct classes of antibiotics were detected in all the batches of the three water brands at densities up to 10^2 CFU/mL. In the whole set of isolates, it was observed resistance against all the antibiotics tested. The most represented resistance phenotype (ATB, BioMerieux and disc diffusion) was resistance against the beta-lactam antibiotics. The antibiotic resistance profiles of the Actinobacteria presented high resistance levels to the antibiotics cefotaxime, meropenem and ciprofloxacin according to minimum inhibitory concentrations (Etest and M.I.C. Evaluator). Bottled mineral water was confirmed as a source of antibiotic resistance mechanisms, with the potential to spread resistance.

TH 143

Important vectors of antibiotic resistance genes in agricultural systems
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2Spreading manure on agricultural soils was recently studied to promote spreading of transferable antibiotic resistance and residual veterinary medicines in agricultural soils. The aim of this study was to compare antibiotic resistance profiles and antibiotic resistance genes in two types of soils: one receiving manure with antibiotics and the other one without. The diversity of antibiotic resistance genes was compared to manure not containing SDZ. In the present study we aimed to characterize plasmids which were captured into E. coli recipients from soil samples of microcosm, mesocosm and field experiments by probing resistance genes. The plasmids were mainly assigned to LowGC and IncP-1 plasmids. The diversity of antibiotic resistance genes carried on these plasmid vectors was remarkable. Our data showed that the selection of bacterial populations carrying sul genes often will co-select a wide range of other antibiotic resistance genes. The diversity of antibiotic resistance gene cassettes reported in IncP-1, the ability to efficiently transfer under soil conditions and the wide host range of IncP-1 plasmids strongly suggest that these plasmids are important vectors for spreading antibiotic resistances in the agro-ecosystem. LowGC plasmids have in contrast to IncP-1 plasmids a much more narrow host range but their relative abundance is one to two orders of magnitude higher, and they are important as putative hosts are emerging pathogens of the genus Acinetobater.

TH 144

Spread of antibiotic resistance determinants in wastewater irrigated soils in the Mèze Valley of Portugal
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We investigated the spread of antibiotic resistance determinants by horizontal gene transfer (HGT) in wastewater (WW)-irrigated soils from the Mèze Valley (60 km north of the city of Portimão, Algarve, Portugal). We selected a WW of Mexican origin treated with sewage from the whole of Portugal. WW contains a huge amount of potentially harmful components such as resistance determinants, pathogens and antibiotic resistances. WW-irrigated soils are a potential reservoir for multiresistant organisms, which might pose risks for field workers and consumers of the agricultural products. In the Mèzequall, fields have been investigated with WW for different time periods, from 1 year up to more than 100 years. We studied the possible correlation between the irrigation time and the concentration and dissemination of resistance genes. Furthermore, we performed soil column experiments with different types of soil (soil irrigated with WW for 100 years and rain-fed soil), to test our hypothesis that WW irrigation leads to spread of resistance genes in soils. Gene transfer scenarios were performed as follows: an Enterococcus faecalis donor harbouring a mobilizable broad host range resistance plasmid labelled with the Green Fluorescent Protein (GFP) and the second non-mobilizable plasmid labelled with the Red Fluorescent Protein (RFP) were added to rain-fed and 100 years-irrigated soil, each in soil columns of 20 cm height and 5 cm diameter. After each 10^5 donors were applied to the columns. The rain-fed soil was irrigated with artificial rainwater and the WW-irrigated soil was irrigated with WW once a week, in total three times. The duration of the experiment was 4 weeks. Samples were taken from the top soil and at the end of the experiment from different heights (0.5, 2.5 cm). During irrigation leachate water was collected and pore water was sampled at 4 heights with suction cups. Bacteria in soil and water which have acquired the mobilizable resistance plasmid via plasmid transfer are detectable by green fluorescent. Donors are identified by their green and red fluorescence. Transfer rates for both types of soil and in water will be presented. Furthermore gfp and rfp will be quantified by real-time PCR. The soil column experiment will help assess the role posed by HGT of resistance determinants in WW-irrigated soil.

TH 145

Development of micro-scale leucine incorporation assay and its application for analysis of co-selected antibiotic resistance patterns in Cu-contaminated soil
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It is well known that Cu selects for Cu tolerance in soil microbial communities, but recently it has been documented that Cu may also co-select for antibiotic resistance. Due to the ongoing Cu accumulation in agricultural soils and the risk of human exposure to soil microorganisms, this co-selection phenomenon deserves further study. Soil is a spatially heterogeneous matrix and we thus asked whether co-selected antibiotic resistance is a general phenomenon in the entire soil matrix or whether this phenomenon can be limited to specific micro-habitats in the same long-term (>85 years) Cu-contaminated soil as used for a previous co-selection study (Berg et al., 2016; Environ Sci Technol 48:8724-8728).

To analyze spatial heterogeneity of community-level tolerance to Cu and antibiotics, we first developed a novel micro-scale [3H]leucine incorporation assay for measuring growth of Cu-selecting isolates from soil. The [3H]leucine incorporation rates in 5-µl soil bacterial suspensions (50 ng of biological [3H]leucine added) were determined. The co-selective performance as compared to the original protocol based on larger sample sizes (Bååth et al., 2001; Soil Biol Biochem 33:1571-1574). When combined with an optimized extraction and soil handling protocol, we were able to perform 96 replicate [3H]leucine incorporation incubations based on a single 5-µg soil sample.

The optimized micro-scale [3H]leucine incorporation assay was subsequently used to measure pollution-induced community tolerance to Cu and co-selection to targeted antibiotic resistances and residual veterinary medicines in Cu-contaminated soil samples. Cu-bioavailability was analyzed by a downscaled version of an existing whole-cell bacterial bioassay sensor (Brandt et al., 2008; Environ Sci Technol 42:3102-3108). A low extent of spatial heterogeneity was found for both Cu tolerance and Cu bioavailability, whereas co-selected antibiotic resistance patterns exhibited a highly heterogeneous distribution (i.e. co-selection in some 5-µg soil spots, but not in others).

Our studies indicate that field exposure to Cu may constitute a persistent selection pressure for the expansion of the soil bacterial resistome, but also that co-selection
Gold ingegnerized nanoparticles (Au NPs): first investigation of toxicological effects in fibroblast cell cultures of cetaceans

TH 147

Aspects of antibiotics resistance in Salmonella spp. isolates

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Salmonella spp., mainly some serovars of Salmonella enterica as Enteritidis or Typhimurium, are etiological agents of salmonellosis, the second common gastrointestinal disease in the industrially developed countries. While most cases do not require antibiotic treatment, it can be necessary for immunosuppressed, elderly or child patients.

The increasing resistance of Salmonella spp. to antibiotics can cause complications in such cases as well as to support the horizontal transfer of antibiotics resistance genes in the microbial community. The development and the acquisition of these genes can occur at different places under different conditions - for example by overproducing antibiotic resistance genes in clinical and medical practice or in wastewater treatment plants, supported by biofilm formation. The aim of this work was to study the different aspects of antibiotic resistance in 50 strains of Salmonella spp. isolated from different sources (clinical and environmental)

The isolates were typed by ERIC-PCR and REP-PCR methods and studied for their plasmids profile. Then they were tested by disc diffusion method for the resistance to 12 antibiotics (amoxicillin, ampicillin, cefotaxime, gentamicin, streptomycin, ciprofloxacin, nalidixic acid, trimethoprim, tetracycline, chloramphenicol, nortefuroxam, kanamycin) in broth dilution assay. The occurrence of the isolates with the resistance to the genes (tetA, tetB, tetC, tetG) was detected by PCR method together with the cluster SGI-1 (Salmonella Genomic Island-1).

The strains resistant minimally to one antibiotics (tetracycline, ampicillin, streptomycin, nalidixic acid, ciprofloxacin or chloramphenicol) were examined for the ability of biofilm formation in microtiter plates in different media (LB, BHI, BHI + 2 % glucose) and at different temperatures (25 °C, 37 °C). This work was supported by the project LD11048 of Ministry of Education, Youth and Sports of Czech Republic.

TH 148

Adaptation of bacteria to ameliorate the metabolic burden of carrying antibiotic resistance genes (a systems biology modeling analysis)

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The increasing resistance of antibiotic bacteria is an important problem, and it is unclear what leads to the relatively high abundance of resistant bacteria in the environment. Mathematical models are useful tools for research and management in this area. However, although many of the mechanisms underlying antibiotic action, resistance, and cost of resistance are understood at the molecular level, models typically describe these processes using system-level parameters (e.g., cost of resistance). This constitutes a disconnect that limits the utility of modeling, which can be predicted from basic molecular biology. Mathematical models for Escherichia coli and tetracycline are developed based on existing models of metabolism, plasmid and antibiotic behavior. The model explicitly accounts for the action (i.e. binding to ribosome) and resistance (tet efflux pump), and the metabolic cost of resistance, including copying the plasmid DNA, transcribing the plasmid genes, and the energy consumption of the efflux pump (i.e. ATP requirement to maintain membrane charge). To investigate the fitness of different strains under various conditions, individual molecular mechanisms were simulated using an agent-based approach. The concept of combining biological modeling (systems biology) and ecological modeling (systems ecology) in this manner is referred to as “systems bioecology”. The model reproduces observations from the literature, including growth rate vs. substrate concentration, intercellular tetracycline concentrations of wildtype and resistant strains, growth rate under various tetracycline concentrations and the response of tetracycline resistance in the human gut to a 5-day tetracycline treatment. The results show that these behaviors can be predicted from molecular mechanisms. The model is then used to investigate the role of potassium uptake by the Tet efflux pump. When this feature is included, a mutation in the endogenous potassium resistance gene caused an increase in the growth rate of the inoculated strain.

TH 157

Toxicity of silver nanoparticles to rainbow trout - a toxicogenomic approach

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Silver (Ag) nanoparticles possess antimicrobial properties which makes them convenient adjuvant in consumer products such as clothes and medical devices. NanoAg could leach out from these products and pervade the our environment with perhaps unsuspected impacts. The purpose of this study was to examine and compare the sublethal effects of nanoAg and dissolved Ag in rainbow trout. Changes in hepatic gene expression were monitored to provide insight on the mode of action of both forms of Ag. Oncorhyncus mykiss were exposed to increasing concentrations of nanoAg (20 nm) and AgNO3 for 96 h at 15°C. The liver were also analyzed with a suite of biochemical markers of toxicity. Gene expression analysis was performed using a DNA microarray consisting of 207 stress-related genes followed by quantitative polymerase chain reaction on a selection of genes for validation. The biochemical markers consisted in the determination of lable zinc, metallothioneins, DNA strand breaks, lipid peroxidation and vitellogenin-like proteins. The analysis of total Ag in the aquarium water revealed that Ag was mostly aggregated where 1 % of the total Ag was truly dissolved. Exposure to both forms of Ag led to significant changes in gene expression for 13 % of tested gene targets. About 12 % of genes responded specifically to nanoAg while 10 % of gene targets were responded specifically to dissolved Ag. The levels of vitellogenin-like proteins and DNA strand breaks were significantly reduced by both forms of Ag but DNA breaks levels were lower with nanoAg and could not be explained by the presence of ionic Ag. Lable zinc and the oxidized fraction of metallothioneins were increased by both forms of Ag but LPO was significantly induced by nanoAg only. Discriminant function analysis revealed that the responses obtained by biochemical markers and a selection of gene expression markers able to discriminate completely (100 %) the effects of both forms of Ag. It was found that genes involved in oxidative stress, protein stability and inflammation responses were able to discriminate between the effects of Ag where nanoAg influenced more genes involved in inflammation and dissolved silver involved more oxidative stress and protein stability.

TH 158

Gold ingenerized nanoparticles (Au NPs): first investigation of toxicological effects in fibroblast cell cultures of cetaceans


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Nanoparticles (NPs) are produced intentionally with the aim of developing new materials that exhibit certain specific properties. These properties are related to at least one of their dimensions, which must be less than 100 nanometers (nm). Their use has been proposed in many fields of science and industry. Several studies exist on the toxicological properties of NPs. Although the various toxicological aspects and the diversity of the NPs assessed are just beginning, many deleterious effects have been documented, particularly in animals. However, no data are available for marine top predators of conservation interest, such as toothed whales (Odontoceti). Gold (Au) NPs, also called gold nanomaterials (NMs), have been increasingly used as drug delivery systems for recent cancer treatments. Recently the photothermal conversion property of Au NPs has attracted a keen interest from the scientific community. In fact the Au NPs that are irradiated by a laser beam can efficiently convert the photon energy to thermal energy, which leads to the destruction of biological cells such as tumor and bacteria. Despite Au NPs are considered non-toxic by several authors, there is a serious lack of information concerning their impact on the life health and the environment. The aim of this study was to evaluate the toxicological effects of the ingengerized Au NPs in fibroblast cell cultures of two free-ranging species of toothed cetaceans: striped dolphin (Stenella coeruleoalba) and bottlenose dolphin (Tursiops truncatus), sampled in Mediterranean Sea by non destructive method represented by subcutaneous biopsy. Genotoxicity of Au NPs in cetacean fibroblasts was detected by the alkaline Comet assay. Immunofluorescence technique was used for qualitative and quantitative analysis of the AuNPs uptake in fibroblasts. The cells were also cultured in presence of the genotoxic DNA breakers 4,4′-nitro-2,2′-propiolabisulfonic acid (NPSB2) and MICA stress-related genes were evaluated as targets of potential indicators of man-made pollutants, and the MICA as toxicological stress marker of the immune system. Finally, the Au NPs capacity to pass through the cellular barriers, was
studies. Supported by: FAPESP, CNPq, CAPES, Fundunesp and Embrapa (AgroNano).

other biomarkers there was no statistics difference between groups and control groups. Our results corroborate with literature, showing low acute toxicity of nano-TiO2 and ultraviolet: an ecotoxicological evaluation.

TH 160
Co-exposure to nano-TiO2 and ultraviolet: an ecotoxicological evaluation
F. Stibany, H. M. Maes, S. Giefers, B. Daniels, B. Deutschmann, F. Hischen, W. Baumgartner, H. T. Ratte, A. Schäffer

TH 161
Uptake and distribution of multiwalled carbon nanotubes in zebrafish Danio rerio

TH 162
Development of a test chamber to enable homogenous aqueous phase dispersions of nanoparticles for testing nanoparticle toxicity in fish

TH 163
Silver nanomaterials are one of the major components of healthcare products. Their environmental fate and ecological risk are not well understood yet. Although it is known that silver ion (Ag+) has toxic effects, it has been discussed whether nano-sized silver has its own toxicity. To elucidate this problem, silver nitrate (AgNO3) has been used for development of a standardized test procedure to enable testing toxicity of homogeneous aqueous dispersions of NPs in fish (e.g., zebrafish Danio rerio) that is repeatable and easily transferable across laboratories. The test system (2-L glass beaker) is designed to maintain homogeneous aqueous dispersions of NPs by stirring, and delivers water containing aqueous NPs to fish held in a semi-isolated chamber. With this we have conducted acute toxicity tests with larval zebrafish to establish 96-h LC50 values for TiO2-NPs, Ag-NPs, and Cu-NPs, and compared to metal ion controls and body length and whole length were affected in SNC. Lipid peroxidation (LPO) and protein carbonylation (PC) were increased in presence of SNC and protein carbonylation and PC were increased with increasing exposure levels, but more investigation is needed.

Further studies will be carried to investigate the relevance of considering the nano-TiO2 in toxicological studies. Supported by: TAFESP, CNPq, CAPES, Funduspan and Embrapa (AgroNano).

TH 164
Salinity- and pH-dependent silver nano-toxicity in post-hatch embryos of Japanese medaka

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Our group has been investigated nano-sized silver toxicology using Japanese medaka embryos and elucidated that silver nanocolloids cause severe inhibition of embryogenesis and morphological effects were moderated in ERM as well as happened in case of NSP. We estimated that embryos in ERM were relieved from biological effects of silver toxicity, which may explain some of the differences in NP toxicity reported in the literature. The objective of this investigation was to develop a standardized test procedure to enable testing toxicity of homogeneous aqueous dispersions of NPs in fish (e.g., zebrafish Danio rerio) that is repeatable and easily transferable across laboratories. The test system (2-L glass beaker) is designed to maintain homogeneous aqueous dispersions of NPs by stirring, and delivers water containing aqueous NPs to fish held in a semi-isolated chamber.

With this we have conducted acute toxicity tests with larval zebrafish to establish 96-h LC50 values for TiO2-NPs, Ag-NPs, and Cu-NPs, and compared to metal ion controls and body length and whole length were affected in SNC. Lipid peroxidation (LPO) and protein carbonylation (PC) were increased in presence of SNC and protein carbonylation and PC were increased with increasing exposure levels, but more investigation is needed.

Further studies will be carried to investigate the relevance of considering the nano-TiO2 in toxicological studies. Supported by: TAFESP, CNPq, CAPES, Funduspan and Embrapa (AgroNano).
Our results indicate that changes in gene expression pattern are elicited by AgNPs, and differences between the two commercial AgNPs were clearly evident. The data activities were enhanced by exposure to increasing QD concentrations, whereas ascorbate peroxidase and glutathione reductase activities were not significantly affected. SOD able to stimulate a comparable PC synthesis. Since the production of PCs is a specific signal of the presence of free metal ions, our findings suggest that QDs were taken up by proteins (bax and bcl-2) were measured. At least three independent experiments were conducted.

Carbon nanospheres, multiwalled carbon nanotubes and β-cyclodextrin polymer as emerging pollutants

The use of engineered nanomaterials is fast growing and the environmental concerns about them are also growing. The nano size (<100 nm) of nanomaterials which has resulted in their vast applications has since raised concerns about their toxicity. Carbon nanomaterials are one of these groups that are being used by many industries. Some of these nanomaterials have been specifically engineered for water purification purposes and hence their fate in the aquatic system is not yet fully understood. There is therefore a need for in vivo studies on these nanomaterials that are known to be good pollution indicators. The pollution indicators chosen for this study were Porcellia reticulata (fish), Daphnia pulex (water flea), Vibrio fischeri (bacteria) and Selenastrum capricornutum (algae) and the carbon-based nanomaterials tested were multi-walled carbon nanotubes (MWCNTs), carbon nanospheres and β-cyclodextrin polymer. These nanomaterials were vastly characterized to determine their physico-chemical properties in their dry and wet state as per the protocols. All the exposures experiments were done in the presence and absence of humic acid (20, 10, 5, 2.5 and 1.25 ppm humic acid concentration) at neutral pH. Particle size and zeta potential were monitored over 96 hr (the longest time for fish exposures). LC50 (lethal concentration) and the NOEC (no observed effect concentration) were identified. 

Elucidated that SNC causes severe inhibition of embryogenesis and morphological changes such as blood clots, percardiovascular edema, tubular hearts, small eyes, shrunken heart, and spinal deformities at 0.5 mg/L and 1.0 mg/L. This study explores the toxic mechanisms of silver nanocolloids (SNC; average particle size 3.6 diameter nm, purity 99.99%, pure water solution) using medaka embryo model. From DNA microarray analyses, 118 genes are up-regulated (>2.0 fold) and 117 genes are down-regulated (<0.5 fold). The rapid increase of nanotechnology has raised the concern about the potential adverse effects of nanoparticles on the ecosystems and living organisms. Quantum dots (QDs) are semiconductor nanocrystals, candidate to be widely used for biomedical applications and microelectronics, due to their unique optical properties. However, their environmental impact and the mechanism of toxicity still have not been fully elucidated. Phytoplankton represents the first link of the food chain in natural waters, thus the aim of this study is to evaluate the biochemical and physiological effects of QD NP in C. maenas and determine NP bioavailability and accumulation in key tissues (e.g. gill and hepatopancreas). After acclimation of 15 days, adult male crabs were exposed in a semi-static test system to 0, 0.1, 1.0 and 10 mg TiO2 N/P (24.5 ± 10.6 nm diameter) and 10 mg/L bulk TiO2 (134.0 ± 42.5 nm), a particle size control. For each treatment 15 crabs (5 per replicate) were evaluated. Daily, crabs were brieﬂy removed from exposure tanks, fed with frozen cockles (Crasostrea edulis) in clean water to avoid dietary TiO2 exposure and 100% exposure media exchanged. After exposure, biochemical responses associated with physiological function will be measured including gill NAR+ ATPase activity, osmotic pressure and major ions in haemolymph as indicators of osmoregulation; oxidized/reduced glutathione ratio and ferric antioxidant status in haemolymph as biomarkers of oxidative stress; pH and lactic acid in haemolymph and oxygen consumption as end points of respiratory metabolism and, ON ratio and total soluble proteins in haemolymph as end points of energetic status. Gill and hepatopancreas will also be analyzed for metal accumulation. Results are under analysis; however obtained information will likely add to our understanding of potential TiO2 NP toxicity in an ecologically important benthic coastal species.

Effect of silver nanoparticles on ovarian follicular cells of marine medaka (Oryzias melastigma) 

TH 165

Siemens nanospheres and target genes analyses in medaka embryos

TH 164

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TH 159
Effects of silver nanoparticles in the freshwater snail Physa acuta
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The environment is constantly being exposed to various types of contaminants due to anthropogenic activities. Recent studies showed an increase in the amount of metals, such as copper, silver or cadmium that can be found in aquatic ecosystems due to industrial effluents and domestic wastewaters disposals. Silver nanoparticles are nowadays widely used in various products, such as cosmetics, pharmaceutical products, cleaning products or even contact lenses, which will lead to an increase in concentration in the environment. Therefore, this study aimed to assess the survival, reproduction and egg viability of the freshwater pulmonate snail Physa acuta after exposure to silver nanoparticles and also its counterpart silver nitrate in laboratory conditions. A 96h exposure test will be used to evaluate acute toxicity and a 14/28 days chronic test will be carried out where egg viability and the number of new born snails will be evaluated. Results showed a decrease in reproduction and egg hatching in higher concentrations. Silver nanoparticles and ions significantly affected all 3 endpoints measured.

This study highlights that silver nanoparticles are bringing new inputs of silver as contaminant (as ions and nanoparticles) into the aquatic ecosystems.

TH 170
Evaluation of chronic toxicity of copper oxide nanoparticles to microcrustacean marine Mysidopsis Juniae
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The copper oxide nanoparticles are components of antifouling paints that are used for painting the hulls of boats. Their decomposition can provide both the soluble ionic forms and nanomaterials, becoming an important source of contamination of aquatic ecosystems. Chronic toxicity studies in aquatic environments is a trend in environmental toxicology, since in many cases the contaminants from this group are highly diluted and do not offer sufficient concentrations to elicit acute effects but capable of causing only long-term effects. This study was aimed at assessing the chronic toxicity of copper oxide nanoparticles on the marine microcrustacean, Mysidopsis Juniae. Tests were conducted with concentrations of 100, 75 and 50 µg.L⁻¹ and 40 test organisms with sexual dimorphism were added in each of the 2 liter tanks. The organisms were exposed for 30 days to the nanoparticles. The test was carried out in semi-static environment and the water exchange occurred once a week. The test conditions were salinity 32; photoperiod 12 hours of light and 12 hours of darkness and temperature 24±1 °C. The mysids were fed daily with Artemia sp. nauplii enriched with omega 3 and cod liver oil. The parameters evaluated were mortality, reproduction and total size. The laboratory mortality test charts for monitoring the sensitivity of test organisms. The data were analyzed with the softwares Statistica 7 and Ministat 16. In order to check the differences between the evaluated parameters Student’s t test with a confidence interval of 95% and the Dunnet test for calculating the NOEC were used. The mysids exposed to nanoparticles suffered changes in number of mortality, fertility and size in concentrations above 50 µg·L⁻¹.

TH 171
Effects of in vivo exposure to TiO2 on Mytilus immune parameters
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Acute toxicity of different engineered nanoparticles (ENP) underlined the potential risk of ENPs exposure to aquatic life. Apart from traditional ecotoxicity testing, more specific assays like immunotoxicity, genotoxicity, oxidative stress, may help understanding the major toxic mechanisms and modes of actions that could be relevant for different ENPs aquatic toxicology.

In this work, we filled those knowledge gaps by investigating sublethal effects of nCeO2 on two aquatic invertebrates, the freshwater mussel Dreissena polymorpha and the freshwater snail Physa acuta. The organisms were exposed for 30 days to the nanoparticles. The test was carried out in semi-static environment and the water exchange occurred once a week. The test conditions were salinity 32; photoperiod 12 hours of light and 12 hours of darkness and temperature 24±1 °C. The mysids were fed daily with Artemia sp. nauplii enriched with omega 3 and cod liver oil. The parameters evaluated were mortality, reproduction and total size. The laboratory mortality test charts for monitoring the sensitivity of test organisms. The data were analyzed with the softwares Statistica 7 and Ministat 16. In order to check the differences between the evaluated parameters Student’s t test with a confidence interval of 95% and the Dunnet test for calculating the NOEC were used. The mysids exposed to nanoparticles suffered changes in number of mortality, fertility and size in concentrations above 50 µg·L⁻¹.

TH 172
Method development to test toxicity of fullerenes (nC60) on sediment-dwelling invertebrate Chironomus riparius larvae
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The possible ecotoxicological risks of nanoparticles (NPs) have received increasing research attention in recent years as a result of the enormous economic potential of nanotechnology-related applications and the lack of information about such risks. The extent to which current standard ecotoxicological methods can be used to accurately measure the ecotoxicological risks of NPs is largely unknown due to the unique chemical properties of nanoparticles which substantially differ from those of hydrophobic organic chemicals and dissolved metals. The aim of this study was to investigate the extent to which fullerenes (nC60) are applied in a new environmentally realistic method to test short-term effects after exposure for 10 d. First, an environmentally realistic method to test fullerenes (nC60) toxicity to benthic organism C. riparius was created by allowing suspended fullerenes to settle down creating a layer on the substrate. Tests were ceased at concentrations of 0.36 to 0.55 mg·cm⁻². This created an environmentally realistic exposure method which simulates a sensitive exposure route for C. riparius given its feeding habits. Second, two different food concentrations (0.5 % and 0.8 % Uricula sp.) in the sediment were tested to investigate the impact of this parameter on toxic response. Thus, we hypothesized that higher food concentrations will decrease toxic effects. In the 0.5 % food level treatments, there were significant differences in mortality and growth rate at the point of controls. Fewer effects were observed for the higher food treatment. Fullerenes agglomerates were observed by electron microscopy in the gut but no absorption into the gut epithelial cells was detected. In the organisms exposed to fullerenes, microvilli were damaged and significantly shorter. The potential toxic effects of fullerenes appear to be caused by morphologic changes, thus inhibiting the larvae growth.

TH 173
Multibiomarker assessment of cerium dioxide nanoparticle (nCeO2) subthalic effects on the freshwater invertebrates Dreissena polymorpha and Gammarus roeseli
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Currently, cerium dioxide (nCeO2) are among the most widely used NPs, mainly as a fuel additive for their catalytic properties. As a result, the release of nCeO2 and the subsequent environmental exposure are predicted to be consequent, while the risks incurred are still unknown. Most of the aquatic ecotoxicological studies published from now have focused on the acute toxicity of nCeO2, often at environmentally irrelevant concentrations, and have shown its relative innocuousness. However, with significant emerging nanotechnology-related applications and the lack of information about such risks, the extent to which current standard ecotoxicological methods can be used to accurately measure the ecotoxicological risks of NPs is largely unknown due to the unique chemical properties of nanoparticles which substantially differ from those of hydrophobic organic chemicals and dissolved metals. The aim of this study was to investigate the extent to which cerium dioxide (nCeO2) are applied in a new environmentally realistic method to test short-term effects after exposure for 10 d. First, an environmentally realistic method to test fullerenes (nC60) toxicity to benthic organism C. riparius was created by allowing suspended fullerenes to settle down creating a layer on the substrate. Tests were ceased at concentrations of 0.36 to 0.55 mg·cm⁻². This created an environmentally realistic exposure method which simulates a sensitive exposure route for C. riparius given its feeding habits. Second, two different food concentrations (0.5 % and 0.8 % Uricula sp.) in the sediment were tested to investigate the impact of this parameter on toxic response. Thus, we hypothesized that higher food concentrations will decrease toxic effects. In the 0.5 % food level treatments, there were significant differences in mortality and growth rate at the point of controls. Fewer effects were observed for the higher food treatment. Fullerenes agglomerates were observed by electron microscopy in the gut but no absorption into the gut epithelial cells was detected. In the organisms exposed to fullerenes, microvilli were damaged and significantly shorter. The potential toxic effects of fullerenes appear to be caused by morphologic changes, thus inhibiting the larvae growth.

TH 174
Effects of TiO2 nanoparticles on Mysidopsis galloprovincialis 48 h-old larvae
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Engineered nanomaterials are at the forefront of ecotoxicologist agendas due to their widespread use in a broad range of industrial and domestic sectors. Actually, they are manufactured in increasing amounts year-by-year. Particularly, nanoscopic titanium dioxide (TiO2) is used by a variety of industries mainly for catalysis and photocatalysis.
Engineered metal nanoparticles (NPs), such as Copper oxide (CuO) NPs are increasingly being used in consumer products, and human and environmental exposure is likely to increase. CuO NPs may cause adverse effects as a result of their small size (<100 nm) and high surface to volume ratio. Engineered metal NPs have been shown to cause organole, DNA damage, oxidative stress, apoptosis, up/down regulation of proteins etc. CuO NPs released into the aquatic environment will likely agglomerate and/or precipitate in the brackish water of the estuary and subsequently accumulate in the sediment compartment. Therefore the benthic environment is a likely site of action (e.g., proteins and DNA) either directly or indirectly via the release of ions or production of reactive oxygen species. In this study we examined dietary uptake, intracellular distribution and genotoxicity of Cu added to sediment in different forms and particle sizes for the deposit feeding polychaete Nereis diversicolor. Cu was added to sediment as aqueous Cu (administered as CuCl2 -2H2O) or as CuO particles of different sizes; NPs (polydispersed-100 nm) and micro particles (5 μm).

Silver nanoparticle dissolution and Ag speciation as key parameters for toxicity of AgNP to algae

Silver nanoparticle dissolution and Ag speciation as key parameters for toxicity of AgNP to algae

TH 175
Cellular and subcellular localization of Au nanoparticles and Au bioaccumulation in mussels exposed to Au nanoparticles of two different sizes
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Sub-cellular localization of nanoparticles in invertebrates has been scarcely studied and the results are inconclusive. Here, mussels, Mytilus galloprovincialis, were exposed for 3 d, to Na+-citrate stabilised Au nanoparticles (NPs) of two different sizes (5 and 40 nm), as well as to bulk and ionic (HAuCl4) Au forms. The nominal exposure concentration for each size was 0.75, 3.75, 5.0, 7.5, 10, 25 and 50 μg Au/l. Au accumulation was quantified in mussels exposed to the different Au forms, as Au in mussel soft tissues was measured by ICP-MS. Tissue Au accumulation was dose dependent for all treatments. Lowest Au concentrations were found in mussels exposed to bulk Au. Au nanoparticle characterization was carried out at the Centre for Ultrastructural Imaging, King’s College London. Work funded by the European Community’s 7th FP (FP7/2007-2013) under grant agreement n° CP-FP 214478-2 and by the Basque Government (GIC07/26-IT-393-07). A. J-R is recipient of a pre-doctoral fellowship from the University of the Basque Country.

TH 176
Metal-particle size affects metal bioaccumulation from sediment and depuration in a deposit-feeding snail C. Banta1, L. Selck1, I. Marigómez1, S. K. Misra2, E. Valsami-Jones3, V. E. Forbes3
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TH 177
The ecotoxicology of engineered metal oxide (ZnO) nanoparticles with respect to the aquatic sediment dweller, Lirincombulus variagatius
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Since the 1990’s there has been a rapid increase in the use of engineered nanomaterials and one of the side effects of this “nano-boom” is the potential for these NPs to be released into the environment. The novelty properties of these NPs (NPs) make them extremely useful in industry however these properties may also render them uniquely hazardous in nature. Zinc oxide NPs are used in a variety of applications including sunscreen, antibacterial lotions, lipsticks, UV stabilisers in plastics, as a catalyst for chemical reactions, as a food additive, as a fuel additive. The potential environmental importance of these NPs is due to their adsorption on to particulate matter which is a key route of entry and can have a significant influence on the toxicity and bioaccumulation of Zn in aquatic ecosystems.
Using an increasing suite of nanoscale tools, the ecotoxicity of silver nanoparticles (AgNPs) in sewage sludge is studied.

The ecotoxicity and behavior of nanoparticles in the environment is currently unknown due to the various effects they can cause on aquatic organisms. The objectives of this study include:

1. Using a variety of in vitro tests to evaluate the ecotoxicity of AgNPs.
2. Using the rainbow trout gonadal cell line RTG-2 as a bioindicator of cell toxicity.
4. Using Daphnia magna to assess the toxicity of AgNPs.
5. Using the simulation of sewage treatment processes (STPs) to evaluate the toxicity of AgNPs.

The results of this study can inform future research on the environmental effects of AgNPs and guide the development of strategies to mitigate their potential risks.

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Polypehuryl fullerene can mitigate toxicity effects of cadmium on yeasts
A. Pradhan, S. Seena, C. Pascoal, F. Cássio

With inception of nanotechnology, nanomaterials are currently in the prime line of research. Polypehuryl fullerene is one of the widely commercialized nanomaterials and applied for technical, medical fields. Unlike fullerene, polyhydroxy fullerene is known to be biocompatible, and recent studies reported its antioxidant properties. On the other hand, cadmium toxicity has been often attributed to the ability of this metal to induce oxidative stress. To assess antioxidant properties of polyhydroxy fullerene, the model yeast Saccharomyces cerevisiae was exposed to cadmium (≤5 ppm, 3 levels) in the presence or absence of polyhydroxy fullerene (≤500 ppm, 3 levels) at different pH (5.8, 6.3, and 6.8). The size distribution and dispersion of polyhydroxyl fullerene in the stock suspension were measured with DLS (x-average 143 nm, Pd 0.365).

Yeast growth, plasma membrane integrity and accumulation of reactive oxygen species (ROS) were investigated in the presence or absence of Cd²⁺ and/or polyhydroxy fullerene. Yeast growth was inhibited up to 34% by exposure to Cd²⁺, but was not affected by polyhydroxy fullerene. At pH 5.8, the exposure to the highest concentration of Cd²⁺ and polyhydroxy fullerene (500 mg L⁻¹) stimulated yeast growth by 59 and 61% at the exponential and late exponential growth phases, respectively. Growth increased even more when pH increased to 6.8. Severe plasma membrane disruption and ROS accumulation were observed after exposure to the highest concentration of Cd²⁺ in the absence of polyhydroxyl fullerene. Membrane disruption and ROS accumulation decreased with increasing concentration of polyhydroxy fullerene and with the increase of pH to 6.8. Overall, results indicated that polyhydroxy fullerene is not toxic to yeasts and may potentially trigger antioxidant defense mechanisms to mitigate metal-induced toxicity.

Genotoxicity of cotton cellulose nanofibers
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Cellulose whiskers have potential for applications in the future. These include optical document security and improvement of the mechanical resistance of thin films containing polyolelectrolytes in lithium batteries, as well as reinforcement in the polymeric matrices of environmentally-friendly wrapping materials and possible use in medical applications. This work evaluates the genotoxicity of cotton cellulose nanofibers intended for application in the field of New nanocomposites. The emergence of proposals for the application of nanomaterials necessitates detailed study of their toxicity, considering their safety in terms of the environment and living beings, as well as possible modifications of production techniques that could reduce any toxicity, where present. Furthermore, the use of these materials in the health sector could improve biocompatibility, which is an obligatory requirement of regulatory agencies. Nanotoxicological studies aim to ensure the safe application of these materials, hence increasing their acceptance and minimizing any future problems that might be associated with their large-scale usage. This work describes the genotoxicity evaluation of different cotton cellulose nanofibers (white cotton, brown cotton, ruby cotton and green cotton) in this study we investigate the genotoxicity by the use of Allium cepa chromosomes aberration test, comet assay, animal cell cytogenetic analysis, clonogenic assay and molecular assay to measure DNA breakdown by the nanomaterials. The results showed that the effects depend on the cotton nanofiber type, being that brown cotton produces more genotoxic effects than others cotton nanofibers.

Modelling health effects from inhalation of nano-objects
A. Laurent,1 M.Z. Hauschild,1 S. Bruder,2 P. Hertleer,1 E. Teixeira,a A. Moroni,1 A. Moccia, A. Marcomini

There have been many attempts to model the life cycle assessment (LCA) and risk assessment (RA) to engineered nanomaterials, i.e. products embedded with nano-objects (NO). However, there are important limitations in modelling capacity for quantifying the exposure, effects of NO currently present LCA and RA of nanomaterials to include the assessment of the environmental and health risks as well as the toxic impacts stemming from the releases of nano-objects to environment. To help abridge some of these gaps, we draw on existing knowledge of the field of nanotechnology to provide an approach to model the health effects from inhalation of NO, with specific focus on TiO₂ and nanosilica. We apply a physiologically-based pharmacokinetic model for nanoparticles (NPs) and a body mass balance of the human body after inhalation. By using data from in vitro and in vivo studies, we establish dose-response relationships, using the formation of reactive oxygen species (ROS) as a response metrics, and relate the concentrations of NO at the target organs to respective cellular responses. This enables to localize where effects occur in the human body and identify where the hazard may arise, based on the hierarchical oxidative stress stress. A valuable data does not enable to translate the cellular responses to a required whole-body response, e.g. incidence of disease. We thus provide recommendations to fill in this gap and we identify important data and modelling components necessary to achieve a full damage modelling; these include the need for specific chronic tests and methods to extrapolate acute in vivo assay results to chronic in effects for which we discuss opportunities for data incorporation into the developed modelling framework. Overall, the proposed approach thus aims to serve as a first step towards a full assessment of the effects of NO on human health. The relevance of the work applies to both risk assessment and LCA fields although the combination of the approach with a consistent (environmental) exposure model is still required to support proper risk and impact assessment from nanomaterials.

Moving towards quantitative ecological risk assessment of engineered nanomaterials
A. Mocci, D.R. Hrostovz, A. Critto, A. Marcomini

With the development of nanotechnology, nanomaterials (NMs) have been introduced into products that will be used in the environment, for example, in clothing, food packaging, cosmetics, and devices for the treatment and collection of water. The toxicity of nanomaterials is still not well understood, and their acceptance and applications are under scrutiny. The impact of engineered nanomaterials (ENMs) on humans and the environment is not well known due to the limited analytical recovery rate of TiO₂ and 2) of the total TiO₂ added is discharged with the outflow of the STP and that more than 95% of the TiO₂ is adsorbed to the activated sludge. Glass fiber filtering of samples indicated that the main part of TiO₂ in the outflow has to be attributed to particles below 0.6 µm. The TiO₂ balance showed a gap of 18% (trial 1) and 37% (trial 2) probably due to the recovery rate of TiO₂ was more than 95% and 90% respectively according to the OECD Guideline 292. In this study, the recovery rate of TiO₂ was 96% and 92% respectively according to the OECD Guideline 292. In this study, the recovery rate of TiO₂ was 96% and 92% respectively according to the OECD Guideline 292. In this study, the recovery rate of TiO₂ was 96% and 92% respectively according to the OECD Guideline 292. However, the lack of data and modelling capacity for quantifying the fate, exposure and effects of NO currently prevents LCA and RA of nanomaterials to include the effects of NO on human health. The relevance of the work applies to both risk assessment and LCA fields although the combination of the approach with a consistent (environmental) exposure model is still required to support proper risk and impact assessment from nanomaterials.

Mass balance of nanoscale titanium dioxide in laboratory wastewater treatment plants according to OECD 303
S. Gartner,1 C. Nickell,2 M. Stanz,3 S. Damme,4 L. Erdinger,1 T.A.J. Kubisbi,5

The mass balance of nanoscale titanium dioxide (TiO₂) in laboratory sewage treatment plants (STP) has been determined as being in the order of 70-85% (Kiser et al. 2009), and 1 g L⁻¹ SMP. The
The application of NZVI to two soil microcosms at a dose of 34 mg g⁻¹ soil efficiently immobilised Pb (25%) and zinc (20%). Exposure to NZVI had little impact on the immobilization of the alkaline soil was more effective for lead than for zinc, regardless of soil pH. The immobilization process in the alkaline soil was increased by 51.0% for the acid soil and 8.3% for the alkaline one. Soil properties were not adversely affected after treatment with nZVI. These results suggest that nanoscale iron particles are very effective for the transformation and detoxification of a wide variety of common environmental contaminants, both organic and inorganic. The introduction of nanoparticles, the use of binding agents containing nanoscale materials has led to a new generation of environmental remediation technologies, which are cost-effective and targeted to some of the most challenging environmental problems.

In this work, nanoscale zero-valent iron (NZVI) nanoparticles have been used as an immobilisation strategy to reduce Pb and Zn availability and mobility in polluted soils. The application of NZVI to two soil microcosms at a dose of 34 mg g⁻¹ soil efficiently immobilised Pb (25%) and zinc (20%). Exposure to NZVI had little impact on the microorganisms of microcosms and plants in vitro. Three bacterial genera (Agrobacterium, Pseudomonas and Serratia) were used as treatment-related biomarkers. These biomarkers ruled out a broad bactericidal effect on the bulk soil microbial community. A transcriptome analysis of the genes did not reveal any changes in their expression ratios after the NZVI treatment: 1.6 (narG), 0.8 (nirS) and 0.7 (gyrA) in the Pb microcosm and 0.6 (narG), 1.2 (nirS) and 0.5 (gyrA) in the Zn microcosm. However, significant changes in the structure and composition of the soil bacteria population were detected by fluorescence in situ hybridisation. Thus, our results showed that NZVI toxicity could be highly dose specific and species dependent, and the effective applicability of the proposed molecular approach in assessing the impact of this immobilization strategy on soil microbial properties.

To study the molecular and cellular basis of silver nanoparticle (AgNP) toxicity, we here used a recently established in vitro model of earthworm coelomocytes in comparison to the conventional in vivo molecular ecotoxicology approach. Compared to the latter, the in vitro test organisms were exposed to NPs of uniform size held in a monodisperse matrix, in vitro models benefit from the ease of controlling exposure conditions in a defined set of biochemical milieus that NPs may encounter. The AgNPs tested in the present study originated from the same source, but to enhance the colloidal stability in the in vitro test media the NPs were pre-treated with serum proteins. The addition of physical characterisation of AgNPs, the active silver ion fraction was measured in serum-supplemented cell culture medium and in soil pore water. Using flow cytometry and atomic absorption spectrophotometry, we show that a specific population of coelomocytes can accumulate AgNPs in vitro and that silver uptake from the soil environment was evident at the organismal level (measured as total accumulated silver).

The use of zero-valent iron nanoparticles (nZVI) to remediate contaminated soil and groundwater (nanoremediation) has gained increasing amounts of attention within the last decade due to its potential for broader application, higher reactivity, and cost-effectiveness compared to conventional in situ methods. The aims of this study were, firstly, to evaluate the efficiency of the remediation with nZVI to immobilize lead and zinc in two soils (acid and alkaline), and, secondly, to determine the influence of this nanoremediation technology on physico-chemical and biological soil properties. The bioavailability of these metals in soils was determined after a sequential extraction procedure, analyzing soluble, exchangeable, and organic complexes linked to reactive sites.

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The objective of this study was to determine the bioactivity of nano-TiO₂ and to assess their toxicity in presence of humic substances (HS) which are natural components of soil necessary for higher plants growth.

In this study we used a metal-containing nanomaterials - sodium-dioxide titanium (nano-TiO₂), which are characterized <75 nm in size of particles in 10 weights % of water suspension, manufactured in the company «Sigma-Aldrich», USA. Nano-TiO₂ represents oxide the titanium (IV), the mix of two known crystal modifications of dioxide titanium - anatase and rutile.

Among HS we have chosen “Pow-Humus”(Le-PhK) (K-humate, originated from leonardite), manufactured by German firm ‘Humintech’.

The adverse effects of NPs cannot be predicted from the bulk material as result of their small size and unique structure which give to nanoparticles very specific properties: chemical, biological and environmental. This study aims to study the effects of ZnO nanoparticles on soil receptors.

Due to its antimicrobial activity, silver nanoparticles (Ag-NPs) are among the most used NPs worldwide, yet little information is available regarding their effects, particularly in soil dwelling organisms. Enchytraeids (Oligochaeta) are important members of the soil fauna which actively contribute to the acceleration of organic matter decomposition and nutrient recycling processes. Hence, for hazard and risk assessment it is important to provide toxicity data for these organisms, and if possible to understand more in regards to the possible action of AgNPs within organisms. To study this we used chemical soil analyses were performed before and after toxicity assays, using different extractants, to determine the distribution of the Zn forms in the different concentration of nanoparticles; 3) humus substances eliminate the toxic effect of nano-TiO₂ in water suspension which inhibits root growth.

Further research is required for the evaluation of humus substances influence on the nanomaterials which are more and more widely distributed in the environments including the natural soils [1; 2].

TH 196
Gene expression profile of Enchytraeus albidus (Oligochaeta) in response to silver nanoparticles and silver nitrate
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While the precise mechanisms of toxicity of nanomaterials are unknown, it is common to extrapolate the derived toxicity data of NPs from in vitro systems to humans, using alternative in vivo models or alternative endpoints (e.g. enzyme activity). The observed differences in toxicity between the two exposure scenarios could not be explained by dissolution of the AgNPs, as measured Ag⁺ activity (ion selective electrode) in the soil water solution.

Acknowledgements: This work was supported by the Spanish project RITA 2010-00018-00-00.

TH 197
Role of p38MAPK in ROS induced DNA damage & apoptosis due to silver nanoparticle exposure in Caenorhabditis elegans and human cell line: comparative toxicity approach
J.C. de la H. Eom, N. Chatterjee, D.Y. Lim, J.M. Ahn

University of Seoul, Seoul, South-Korea (Rej)

Our approach is to investigate the role of p38MAPK as in vivo model system, we conducted comparative toxicity of silver nanoparticles (AgNPs). In our previous study, p38 MAPK mediated oxidative stress was observed as a toxicity mechanism in C.elegans and human lymphoma cell, Jurkat. In the present study, we find out the link between oxidative stress, DNA damage & apoptosis in C.elegans and human lymphoma cell, Jurkat. We hypothesised that the stress responsive protein, p38MAPK (PK-1, a homologue in C.elegans) is conserved and unavoidable for toxic responses due to AgNPs exposure. Our approach is to investigate the role of p38MAPK as in vivo model system, we conducted comparative toxicity of silver nanoparticles (AgNPs). In our previous study, p38 MAPK mediated oxidative stress was observed as a toxicity mechanism in C.elegans and human lymphoma cell, Jurkat. We hypothesised that the stress responsive protein, p38MAPK (PK-1, a homologue in C.elegans) is conserved and unavoidable for toxic responses due to AgNPs exposure. Our approach is to investigate the role of p38MAPK in oxidative stress, DNA damage & apoptosis by using p38 siRNA knock down in mammalian in vitro system and human lymphoma cell line.

In addition chemical soil analyses were performed before and after toxicity assays, using different extractants, to determine the distribution of the Zn forms in the different soil samples. This study was funded by the Spanish project RITA 2010-00018-00-00.

TH 199
Mixture toxicity of nano-TiO₂ and ingredients of personal care products
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In the last years many studies have focused on the ecotoxicology of nano-TiO₂. But only a few studies have investigated the mixture toxicity of nano-TiO₂ and organic contaminants. Mixture toxicity has to be considered during risk assessment of nano-TiO₂, because both substance groups often have the same entry pathway into the environment. Nano-TiO₂ and biocides are both ingredients of personal care products. Consequently, they have the potential to be released into waste water treatment plants and subsequently through biosolid amendment into soils.

When treating sewage sludge with raw sewage, a bacterial agent used in personal care and household products. This study was identified as an emerging contaminant in biosolids and subsequently in biosolid treated soils.

In this study the mixture toxicity of nano-TiO₂ and TCC to terrestrial organisms is examined. The acute and chronic toxicity of nano-TiO₂ (10-1000 mg/kg dw) and TCC (12 mg/kg dw) as well as a mixture of both (TCC: 42-675 mg/kg dw together with either 400 or 1000 mg/kg dw of nano-TiO₂) to Eseifiela fetida is tested in accordance with OECD 207 (1984) and OECD 224 (2002). TCC is analyzed in soil samples and worm tissues using liquid chromatography tandem mass spectrometry (LC-MS/MS).

No effect was observed for E. fetida exposed to TCC. However in the acute toxicity test TCC decreased the biomass of E. fetida in a dose dependent manner up to 20% after 14 days. In our study TCC showed a lower acute toxicity to E. fetida as found in a study by Snyder et al. (2011, LC₅₀ 40 mg/kg). Nano-TiO₂ did not show any acute toxicity to E. fetida up to 1000 mg/kg dw. Further tests will show if the simultaneous exposure of E. fetida to both substances will lead to mixture toxicity on basis of acute or chronic toxicity. Results will be presented in the poster presentation.

The investigation of mixture toxicity of nanomaterials and organic contaminants is important because only little is known about potential interactions and the related consequences for e.g. bioavailability or formation of toxic metabolites.

TH 200
The influence of soil pH on the toxicity of ZnO nanoparticles to the isopod Porcellio dearius prunusoid

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The stability of nanoparticles (NPs) is known to be affected by soil properties, like pH, which can change NP surface charge and zeta potential. Consequently, soil pH influences the mobility and bioavailability of NPs. In the present study, effects of NPs and non-nano sized particles, and ZnCl₂ were studied in the isoped Porcellionides pruinosis upon exposure in a forest soil with different pH values. The forest soil presented a pH of 4.5 and was amended with calcium carbonate (1 w/w %), in order to achieve an extra soil pH of 7.3. Soil was then spiked with ZnO NPs (30nm), non-nano ZnO (200nm) or ZnCl₂. Initial results with two TiO₂ NPs, one with and one without coating, with concentrations between 200 mg/l and 1 mg/l - indicate that the different particles show similar effects on R. trifolii. Applied at concentrations between 10 mg/l and 1 mg/l, similar to those possibly used in practice, the two TiO₂ NP did not show any effects on R. trifolii growth as compared to controls without NP. Our further objective will be to test possible effects of NP on two crops (clover and wheat) and on two widespread groups of beneficial soil microorganisms, phosphatase and arbuscular mycorrhizal fungi.

Our results show that - independent from any possible detoxification due to the conversion of silver nanoparticles in the sewage treatment plant to argentite - an unknown factor may be responsible for the toxicity of silver nanoparticles to earthworms. The toxicity of NPs seemed to be influenced in a different way from the correspondent ionic Zn exposure based on ZnCl₂ salt.

This study was financed by the German government (BMBF) and by the EU (FBI). The scientific support of C. Menz, C. Dehnicke, M. Bruns, A. Strobl, M. Schiavo, M. Boeckmann, H. Kandeler, and Prof. H. Käbach are gratefully acknowledged. The authors declare no conflicts of interest.

Experimental Set-up

Two different TiO₂ nanoparticles on growth of Rhizobium trifolii in liquid culture

J. Moll, A. Gogos, M.G.A. van der Heijden, T. Bucheli, F. Widmer

The objective of further experiments will be to assess possible effects of NP on two crops (clover and wheat) and on two widespread groups of beneficial soil microorganisms, phosphatase and arbuscular mycorrhizal fungi.

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concentrations of LC0.001, LC0.01, LC0.1, LC1, LC5, LC10, and LC20, with distilled water as the negative control. MGF CV measurements in the earthworm A. caliginosa (used to monitor soil pollution) and AgNP and AgNO3 solutions were renewed every 3 h and CV measurements taken at hourly intervals for 7 h. Based on the results of these studies and our experience with P-glycoprotein (P-gp) transporters in oligochaetes (not reported here), it is proposed that the oligochaetes adapt to excessive exposure of AgNP and to a lesser extent AgNO3 by the elimination of chemicals via P-gp transporters. In conclusion, MGF CV measurements in the earthworm A. caliginosa (used to monitor soil pollution) and to a lesser extent AgNO3 (used to monitor aquatic toxicity) can be used as a non-invasive, sensitive, and early-warning biomarker of exposure to NP but interpretation of chronic exposure requires further evaluation in view of the role of P-gp transporters in the excretion of excess chemicals from the body.

TH 206
A weight of evidence approach for occupational risk assessment of engineered nanomaterials

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It has been recognized that substantial uncertainties and limitations make the conventional RA infeasible to apply to ENMs today, which leaves regulators with little support in their decision-making. In this context, gaps have been gradually filled by new guidance and recommended practices. At early stages, the small size and moves of ENMs through the decades [1], while quantitative risk assessment results are needed to support near-term risk management actions and regulatory decisions. In response to this need, a number of methodologies and tools to assess the risks from ENMs, despite the limitations, have been proposed. Most of them, however, are not intended to facilitate regulatory decision making. The decision is taken to be predictable-based on current knowledge and experience. As a result, less definitive approach, such as toxicodynamic studies, are likely to be required to support proper risk management actions [1]. In this context a quantitative model for occupational RA and prioritization of ENMs, including probabilistic uncertainty assessment within the ENPRA project. Based on the conventional RA paradigm, it uses effects dose-response data and exposure measurements to rank and prioritize nanomaterials for further testing (in a lower tier) and qualitatively estimate occupational human health risks (in a higher tier). In order to ensure optimal integration of all available data our approaches implement some non-conventional tools like the Weight of Evidence and the Multi Criteria Decision Analysis. The model has been applied to a number of commercially available ENMs considered as case study in the ENPRA project, i.e., titanium dioxide, zinc oxide, silver and multi-walled carbon nanotubes. In order to test its stability and ensure the robustness of the assessment results, probabilistic sensitivity analyses has been performed using the Monte Carlo approach. Referenced papers: [1] Hristozov D, Gottardo S, Critto A, Marcocini A. Risk assessment of engineered nanomaterials: a review of available data and approaches from a regulatory perspective. Nanotoxicology (in press). Acknowledgement - The authors thank the EU FP7-funded ENPRA project

TH 207
Toxicity assessment of nanoclay Clay2, on human hepatic cell line HepG2

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Nanoparticles (NPs) attract a great deal of attention due to their unique properties. Their high surface to volume ratios, specific chemical composition, surface structure and solubility, shape and aggregation results in materials that are qualitatively different from their bulk counterparts. These properties make them suitable for numerous applications ranging from existing and emerging technologies. The most promising use of NPs in the food industry are in food packaging. Dispersion of NPs in polymers increases the barrier function of plastics by creating a tortuous pathway for diffusing molecules, increasing shelf life and nutritional quality of food. Having these advantages, using NPs in food packaging seems inevitable. Since many packaging materials is wasted carelessly, a huge amount of these nanoparticles containing compounds might end up in the environment. As we know, the production and disposal of food produced can also lead to hazards connected with NPs. At smaller micro and nanos scales, properties change significantly, which includes a possible increased toxicological risk. Therefore, each nanoparticle requires its own risk assessment. In this work, the main objective was to study the in vitro effects of a newly developed nanoclay, Clay2 which is meant for use in food packaging, in order to understand the mechanism of action causing toxicity. A range of concentrations of Clay 2 (0-150 µg/mL) have been investigated in the human liver cell line (HepG2). Different assays (total protein content, neutral red uptake, and MTS reduction), were performed, and the results showed a reduction of cell viability with an EC50 of 89 µg/mL. Moreover, oxidative stress, genotoxicity and inflammation responses were explored. Acknowledgement: The authors wish to thank Junta de Andalucía (AGR9896) and Ministerio de Ciencia e Innovación (AGL2010-21210) for the financial support

TH 208
Characterization of aqueous metal nanoparticles and assessment of exposure on immune cell viability and effector function

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The use of nanomaterials is spreading in commercial and industrial activities. However, the interactions between these products and living systems is largely unknown. Particularly, the assessment of environment and human risks are lagging far behind the continuous development of new engineered nanomaterials, while literature data suggest that these materials represent potential environmental and health hazards. We studied the impact of nanostructured composites on human cells, by evaluations of various kinds of toxic effects and gene expression profile by DNA microarray analysis. Cells have been incubated with two different nanomaterials, which are possible candidates to develop new nanocomposites such as Cloisite and Carbon Nanotubes (CNTs). The experiments have been carried out on HaCaT cells, representing an in vitro model of human epidermis. The analyses have been carried out also on a mixture of Cloisite and Carbon Nanotubes. Cytotoxicity analysis of this combination on HaCaT cells is represented in Fig. 2. Different assays (total protein content, neutral red uptake, and MTS reduction), were performed, and the results showed a reduction of cell viability with an EC50 of 89 µg/mL. Moreover, oxidative stress, genotoxicity and inflammation responses were explored. Acknowledgement: The authors wish to thank Junta de Andalucía (AGR9896) and Ministerio de Ciencia e Innovación (AGL2010-21210) for the financial support.

TH 209
Genotoxic and cellular effects of nanoparticles on human cells

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The use of nanomaterials is spreading in commercial and industrial activities. However, the interactions between these products and living systems is largely unknown. Particularly, the assessment of environment and human risks are lagging far behind the continuous development of new engineered nanomaterials, while literature data suggest that these materials represent potential environmental and health hazards. We studied the impact of nanostructured composites on human cells, by evaluations of various kinds of toxic effects and gene expression profile by DNA microarray analysis. Cells have been incubated with two different nanomaterials, which are possible candidates to develop new nanocomposites such as Cloisite and Carbon Nanotubes (CNTs). The experiments have been carried out on HaCaT cells, representing an in vitro model of human epidermis. The analyses have been carried out also on a mixture of Cloisite and Carbon Nanotubes. Cytotoxicity analysis of this combination on HaCaT cells is represented in Fig. 2. Different assays (total protein content, neutral red uptake, and MTS reduction), were performed, and the results showed a reduction of cell viability with an EC50 of 89 µg/mL. Moreover, oxidative stress, genotoxicity and inflammation responses were explored. Acknowledgement: The authors wish to thank Junta de Andalucía (AGR9896) and Ministerio de Ciencia e Innovación (AGL2010-21210) for the financial support.

TH 210
The basic safety assessment of fullerene C60 after oral administration

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Summary: More recently, the development of nanomaterials with particle sizes below 100 nm is promoted extensively. These nanomaterials have already been used in various applications such as foods, medicines and cosmetics, and become essential to our daily life. Especially, fullerene C60 is one of the most promising nanomaterials as foods and medicines because of the unique chemical and physical properties. However, knowledge concerning the potential safety of fullerene C60 on human health and the environment is still fragmentary. Therefore, safety assessment of fullerene C60, following oral administration, is very important for safety assessment of food environment. Here, we...
examined the safety of fullerene C60 by oral administration in mice. BALB/c mice were orally treated with 50 mg/mouse of polyvinylpyrrolidone (PVP)-enwrapped fullerene C60 (PVP-fullerene C60), which is water-soluble fullerene, once daily for 7 consecutive days. During exposure of PVP-fullerene C60, the mice were weighted. No statistically significant differences were noted in mean body weights in the PVP-fullerene C60-treated group when compared to the control group. To further evaluate the safety of PVP-fullerene C60, we examined the hematological parameters and plasma biochemical parameters. No significant hematological change was observed in PVP-fullerene C60-treated mice. In addition, PVP-fullerene C60-treated mouse have little change in the plasma levels of liver injury parameter including ALT and AST, and renal injury parameter including BUN compared to those of control mouse. These results suggest that PVP-fullerene C60 would have no effect in oral acute high dose administration and indicate that the PVP-fullerene C60 could be safe nanomaterials for oral administration. We believe that these data provide basic information that should help to create safe and effective forms of fullerene C60.

Acknowledgement: This study was supported in part by Health Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan.

TH 211

In vitro effects of amorphous nanoisocell particles on the bone metabolism


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Summary: Recently, applications of amorphous nanoisocell particles with a controlled particle size below 100 nm (nSPs) have been increasing in oral care products. nSPs exert various beneficial functions as a polishing agent and/or as a remineralization promoter for tooth, based on its unique physicochemical properties. Because high concentration and frequent use of oral care products containing nSPs in in vitro test, tooth paste, and we use them everyday, we are not able to avoid oral exposure of nSPs in living environment. Accordingly, there are growing concerns about the possibility that unique physicochemical properties of nSPs induced health risks. However, there is little information about adverse effects of nSPs in the context of oral exposure. Therefore, in this study, we tried to evaluate the biological effects on alveolar bone metabolism, because periodontal tissue including teeth is one of the most important organs in oral cavity. Using in vitro osteoclast differentiation model, RAW264.7 cells treated with receptor activator of nuclear factor - ligand (RANKL), osteoclast differentiation was assessed by tartrate-resistant acid phosphatase (TRAP) staining and activity. As the results, it was revealed that at doses that did not induce cytotoxicity by nSPs, TRAP activity and the number of osteoclast cells (TRAP-positive multinucleated cells) by nSPs treatment were equivalent to non-treatment group in the presence of RANKL. Collectively, the results suggested that nSPs exerts no effects on RANKL-induced osteoclast differentiation of RAW264.7 cell at the dose of this study. Now, we are examining the effect of nSPs osteoclast differentiation as next step to evaluate effects on bone metabolism. We believe that applications of nSPs will extend to clinical use in the future. In this study, we performed an in vitro study and the future consideration animal welfare.

Acknowledgement: This study was supported in part by Health Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan.


TH 212

Surface interaction of coagulation factor and amorphous nanoisocell particles plays critical role for acute toxicity


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Summary: Recently, the development of nanomaterials (NMs) with particle sizes below 100 nm is promoted extensively. These NMs have been already used in various applications such as cosmetics and foods. Thus, the exposure against NMs is unavoidable for us in our living environment. Under this circumstance, there is increasing concern about the potential health risks by the unique mechanical properties of NMs. In most cases, however, the safety evaluation of NMs has been insufficient for ensuring their safety. In this regards, using amorphous nanoisocell particles (nSPs), we are studying nSPs-induced hazard identification and qualitative evaluation of exposure level, such as in vivo intravitro distribution analysis by transmission electron microscopy. In previous study, we examined in vivo distribution of dermal administration of nSPs with particle sizes below 100 nm, because NMs were used in cosmetic products. As a result, nSPs with particle size 70 nm (nSP70) penetrate skin barrier and move to bloodstream after dermal application. These findings mean that it is essential to identify the biological effects after systemic exposure to NPs. In this study, we examine the biological effects after intravenous injection of NPs. BALB/c mice were intravenously injected with nSPs of sizes 70, 300, 1000 nm and then assessed for survival, blood biochemistry and coagulation. As a result, injection of nSP70 caused fatal toxicity, liver damage, and platelet depletion, suggesting that nSP70 caused consumptive coagulopathy. Additionally, nSP70 exerts procoagulant activity in vitro, which was diminished in Factor XII-deficient plasma. Collectively, we revealed that interaction between nSP70 and intrinsic coagulation factors such as Factor XII were related to nSP70-induced harmful effects. In other word, it is suggested that if interaction between nSP70 and intrinsic coagulation factors can be suppressed, the safety of nSP70 may be ensured. For example, the safety of nSP70 may be ensured. For example, the safety of nSP70 may be ensured due to reduced effective dose of nSP70 or reduced exposure level of nSP70 in the realistic exposure pathway, such as oral or nasal route. These results would be useful for the safety/risk assessment and evaluation of NMs. 

Acknowledgement: This study was supported in part by Health Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan.

TH 213

Nanofate and assessment of nanotoxicity in the environment - Initial findings from phase 1 of the NanoFATE project

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This presentation will outline the main findings from the first 2 years of the EU FP7 project NanoFATE. The NanoFATE project was conceived to fill knowledge and methodology gaps currently prevailing sound assessment of environmental risks posed by engineered nanomaterials (ENPs). Our vision is to assess environmental ENP fate and risk in example high-volume products for which recycling is not an option, namely; fuel additive, personal care and antibacterial products. Two market ENPs from each production stage were selected, i.e. size, surface properties and varying toxicity. Toxicity of the varying ENPs was being followed through their post-production life cycles i.e. from environmental entry as ‘spent product’, through waste treatment to final environmental and toxic potential effects. This will test the applicability of current fate and risk assessment methods and identify improvements required for assessment of ENPs at an early stage. NanoFATE is established as a systematic understanding of fate and mechanisms of effects in a core set of ENPs and addressing how these may affect the application of current tools for ecological risk assessment. The ENPs we study are associated with commonly and widely used products to provide environmental and economic relevance to our work. Furthermore, the selected ENPs have different core and surface chemistry and physical properties allowing us to elaborate on current understanding of how ENP influence fate and behavior in the environment, and their potential toxicity. This testing of how classical ecotox and risk assessment tools may need modifying to be “nano-applicable” will be achieved by systematically studying aspects that are related to ENP fate and toxicity and seeking to provide the knowledge needed to refine risk assessment tools and practices for use with ENPs.

The presentation given will present the main results and conclusions reached and highlight the lessons learnt.

TH 214

Establishing a comprehensive knowledge base on engineered nanomaterials - the project DaNa

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DaNa project is funded by the German Ministry of Education and Research (BMBF) and supported by the Swiss government. The DaNa project (funded by the German Ministry of Education and Research (BMBF) and supported by the Swiss government) steps in, aiming at a comprehensive presentation of relevant material and toxicology data for nanomaterials.

Further, the DaNa-project acts as an umbrella project aiming at collecting scientific results of recent and current BMBF-projects funded in the field of nanotoxicology and nanomaterials science. The knowledge integrated into the database could also contribute to the prioritisation of further research needs. This information is provided in the DaNa knowledge base, accessible via the internet database, www.nanobjects.info. Data regarding application, material properties, exposure as well as human and environmental toxicology is included. From the pool of data short consumer-oriented articles are generated and published. To make the knowledge base easy to access by the user, the information is organised either according to the different types of nanomaterials or via relevant applications. Additionally, there is a list of publications and links to various websites, as well as a frequently asked questions and FAQs. In a first step, all data and results are evaluated regarding their scientific value applying the DaNa criteria checklist, which demands specification on particle properties and behaviour as well as on toxicological tests. Besides the scientific community, we also address the interested public e.g. journalists, students, scientists from other fields or consumers. An understandable presentation of complex scientific data is ensured. An interdisciplinary group of scientists from biology, toxicology, ecotoxicology, physics and chemistry is engaged to achieve this evaluation and presentation for a broad audience.
TH 241

Setting most robust effluent level against severe uncertainty: application of information-gap decision theory to chemical management

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In the ecological risk assessment, we have to make decisions based on many kinds of imperfect knowledge. For example, when we set limitations for point sources, there may be an uncertainty in relationship between a chemical concentration in effluent and that in environment, and hence it may be difficult to predict an environmental concentration using the information about effluent. Another reason is that the environmental quality criteria to determine the ecosystems, but the ecosystems are much more complicated and the criteria may not protect the ecosystems effectively. We apply information-gap decision theory to set effluent limitations for point sources based on scientific understandings rather than based on groundless predictions even under severe uncertainty. Information-gap decision theory derives the decision that is most robust to uncertainty, by guaranteeing an acceptable outcome under the degree of uncertainty without requiring information about the extent of parameter uncertainty at all. We illustrate the application of information-gap decision theory to derive a general framework to set effluent limitations of pollutants for point sources incorporating cost of reduction in chemical use and cost to wildlife species that are affected by pollutants. Our framework enables us to derive decisions to deal with severe uncertainty in ecological risk management of chemicals.

TH 242

Does probabilistic approach relevant to derive Health occupational exposure limits?

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Current methods for risk assessment are mostly 'deterministic'. They means they treat factors such as the toxicity of chemicals as if they were fixed, and precisely known. But in the real world, factors such as toxicity is not fixed but variable. Therefore, toxicity is measured for only a very small number of species, so scientists have to estimate toxicities to humans and to extend to all the other species that we want to protect.

Current methods for risk assessment try to allow for variability and uncertainty by using 'fixed safety factors', but this fails to give a complete description of the full range of the possible risks. Also, it is difficult to decide how big the factors safety should be.

Probabilistic approaches enable variance and uncertainty to be quantified, mainly by using distributions instead of fixed values in risk assessment. A distribution describes the range of possible values (e.g. for toxicity), and shows which values within the range are most likely. The result of a probabilistic risk assessment can also be shown as a distribution, showing how much within the effect range are most likely. This should provide a better basis for making decisions about chemical risks, because the full range of possible outcomes can be taken into account.

For the first time to our knowledge this approach was utilised to derive Occupational exposure limits professional (OEL). OELs for chemicals are important, as they allow scientists to estimate the concentration of toxic substances in the environment. In this study, we investigated how much of a difference in assessing ecological risk between effects of organism-level and population-level. To get a rough answer to this concern, this study performs a comparative study on the difference between the derived reference values (RPCEs) for risk calculation from approaches of organism-level and population-level. To derive the reference value defined as Clambat-1 for use in population-level ERA, extrapolation approach (Lin and Meng, 2009) using available acute (LC50 or EC50) and chronic (NOEC) toxicity test data is employed. From the comparative results of selected chemicals, a range of differences will be shown and discussed. This study is expected to be helpful in bridging the gap between the protection goals and practical ecological risk assessment.

TH 243

Calibration of hazard quotient trigger values for pesticide risk assessment for non-target arthropods

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The European food safety authority (EFSA) recently developed a methodology to define Specific protection goals (SPGs) for the ecotoxicological risk assessment of pesticides (EFSA, 2010). The next step is the definition of specific protection goals for groups of non-target organisms in consultation with risk managers. It needs to be investigated whether the current risk assessment achieves an adequate level of protection to address the SPGs. According to EFSA (2010) "for each key driver (taxonomic group or other ecological factor), a reference tier should be identified, based on the most sophisticated experimental or modeling risk assessment method currently available that addresses the specific protection goal. This reference tier will then be used to calibrate lower tiers using simpler methods that are practical for routine use." To apply this concept for the group of Non-Target Arthropods (NTAs), we compare the hazard quotients, which currently decide whether a higher tier risk assessment for NTAs should be performed, with the derived reference values for the higher tier risk assessment taking into account the new SPGs. To do so we published data from Draft assessment reports that have recently been summarized by EFSA in a database. Results will be presented at the conference.

References:

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How much of a difference in assessing ecological risk between effects of organism-level and population-level?

B.L. Lin

Most protection goals stated in environmental regulations or policies are aimed at the population level or higher. A movement toward population-level ecological risk assessment (ERA) is gaining acceptance for support of chemical management in recent years. However, due to the reason that population-level ERA is a data-intensive analysis (require data of more ecotoxicity data than organism-level), assessing the ecological risk of a new chemical is mainly based on a limited number of measures of organism-level effects in laboratory toxicity tests. Thereby, there is an enormous concern in understanding how much of the difference in assessing the ecological risk between effects of organism-level and population-level. To get a rough answer to this concern, this study performs a comparative study on the difference between the derived reference values (RPCEs) for risk calculation from approaches of organism-level and population-level. To derive the reference value defined as Clambat-1 for use in population-level ERA, extrapolation approach (Lin and Meng, 2009) using available acute (LC50 or EC50) and chronic (NOEC) toxicity test data is employed. From the comparative results of selected chemicals, a range of differences will be shown and discussed. This study is expected to be helpful in bridging the gap between the protection goals and practical ecological risk assessment.

TH 245

Do we need modelling for a conservative risk assessment? An investigation on Daphnia magna populations

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In this study, we use an individual-based model (IDamP) coupled to a TK/ID module (GUTS) to assess the risk of exposure of Daphnia magna individuals to a toxicant with several mechanisms of action (Dispersogen A), on the population level. Dispersogen A acts on reproduction by increasing the brood size at the expense of neonate length which decreases. It also affects survival, in addition to several other effects measured at higher concentrations. Effects on reproduction (neonate length and brood size) were calibrated using both chronics, GUTS datasets. Both assumptions, stochastic death (SD) and individual tolerance (IT) were tested. Validation was done using data from population tests describing population dynamics at six levels of contamination. The model was more successful in predicting population dynamics using SD approach rather than IT. The promoted reproductive effects (increase in brood size) caused and enforced by legislation to protect occupational safety and health. It can be a tool in risk assessment and in the management of activities involving handling of dangerous substances. For each key driver (taxonomic group or other ecological factor), a reference tier should be identified, based on the most sophisticated experimental or modeling risk assessment method currently available that addresses the specific protection goal. This reference tier will then be used to calibrate lower tiers using simpler methods that are practical for routine use. To apply this concept for the group of Non-Target Arthropods (NTAs), we compare the hazard quotients, which currently decide whether a higher tier risk assessment for NTAs should be performed, with the derived reference values for the higher tier risk assessment taking into account the new SPGs. To do so we published data from Draft assessment reports that have recently been summarized by EFSA in a database. Results will be presented at the conference.
Lepidoptera showed a vulnerability towards different pesticides. Especially in smaller field margins pesticide inputs can lead to reduced abundances of Lepidoptera. These

due to overspray and spray drift as a consequence of the adjoining in-field applications. Our research project focused on the assessment of field margins in an agricultural

tradeoffs can be done for the defined number of field margins. It is likely that the toxic pressure is greater for the metals Cu, Pb, and Zn from 360 locations in the Netherlands covering all major Dutch soil types.

Caterpillars and protection goals: the role of field margins as habitats and the effects of pesticide applications

Bird communities in citrus are influenced by a range of habitat features, such as the characteristics of adjacent areas. Reproductive success of birds in the field is affected by

service value) associated with the implementation of various remedial alternatives based upon prescribed protection goals. In this regard, a formal quantification of the effect

TH 247

due to overspray and spray drift as a consequence of the adjoining in-field applications. Our research project focused on the assessment of field margins in an agricultural

TH 248

improving exposure scenario definitions within REACH: a comparative study of sources to aggregate exposure in Korea and in Denmark

TH 249

Birds' community monitoring in chlorpyrifos-treated citrus. Results of years 1-2 of 3 year program in Valencia region

TH 250

Caterpillars and protection goals: the role of field margins as habitats and the effects of pesticide applications

TH 251

Development of a "hair tube" method to monitor potential effects of plant protection products on small mammal populations - application to a fungicide use

TH 247

Do threshold values for metal concentrations in soil protect the soil ecosystem properly?

TH 248

Improving exposure scenario definitions within REACH: a comparative study of sources to aggregate exposure in Korea and in Denmark

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Caterpillars and protection goals: the role of field margins as habitats and the effects of pesticide applications

TH 250

Birds' community monitoring in chlorpyrifos-treated citrus. Results of years 1-2 of 3 year program in Valencia region

TH 251

Development of a "hair tube" method to monitor potential effects of plant protection products on small mammal populations - application to a fungicide use
Since 2005, an increase of requests of post-registration monitoring studies on e.g. birds, mammals, or bees is observed for plant protection products (PPP) newly (re-)evaluated in Europe. In order to verify in practice the appropriate implementation of risk mitigation measures and get additional information on potential effects of the PPP at population level. As specified in the recently published guidance document based on the EFSA document (2009) concerning bird and mammals, all information coming from post-registration surveillance or monitoring can be used to reduce the uncertainty about the level of protection provided by TER trigger values. However, at present, when post-registration monitoring studies are requested for such organisms, no standard guidelines or methods exist.

Regarding small mammals, a large spectrum of methods has been applied in a variety of published studies, but these are not necessarily applicable to study the dynamic of the populations of different species living in croplands. In this context, a simple and innovative field technique using hair tubes (Suckling, 1977) was further developed and applied to assess effects of a fungicide treatment on small mammal populations in cereal fields. It aimed to monitor small mammals under large scale field conditions by assessing the proportion of hair tubes visited by small mammal species ('hair index') living in cereal fields treated or untreated with the fungicide. Part of the work was to adapt this method to allow the tooing and attempted to calibrate this method against a live-trapping method. Then, the ability to detect differences of population size between fields with different treatment regimes, under a range of different sampling strategies had to be assessed using power analyses. Finally, the hair index was estimated in cereal fields of Seine-et-Marne over a two-year period and the scale of monitoring needed to detect significant changes in abundance and composition of rodents and inverteous mammals on a short-term period was determined. The results demonstrate that this method is a very promising tool able to assess small mammal’s relative abundance at large scale (i.e. in 30-100 fields).

**TH 252**

Screening metal impacts from tissue residues for a calibrated biomonitor using DYM BAM and monitoring data in 6 Luxembourgish rivers

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Metals remain a pollution source for most rivers although they have been pushed from the limelight by emerging compounds. The exposure of river biota to metals via food is rarely evaluated in routine risk assessments. We took the opportunity of recent development on the toxicokinetic model DYM BAM and the calibrated biomonitor for hydropsyche to evaluate the metal pressure in Luxembourgish rivers. We used extensive data on suspended matter that had been collected in the years 2002-2003 to simulate the evolution of tissue residues for hydropsyche assuming that it feeds on suspended matter (hydropsyche is a net spinning filterer). As the uptake and elimination constants for this species are only partially known we used ranges of reported values for uptake from the water phase, ingestion rates, assimilation efficiency as well as suspended matter concentration and elimination to run an uncertainty analysis in STELLA with the DYM BAM equation for 6 differently polluted sites in Luxembourg. Results showed the dynamic of internal concentrations for the biomonitor over a life cycle (July-June of next year) with a decrease over the winter period. Nevertheless, most sites yielded exposures to particulate metals which exceeded internal concentration thresholds for metal-sensitive species. The uncertainty of determining metal concentrations of low-flow suspended matter has a strong influence on the results. As for the DYM BAM parameters: uptake from the water phase had a negligible part in tissue residues while ingestion rate and in particular elimination rates where governing the concentration of metals in an isolated way. While in most of the sites classical organic pollution and eutrophication might dominate the depression of sensitive species the additive impact of metals cannot be ruled out following our calculations.

**TH 253**

Active monitoring programs: useful tools for monitoring effects of some plant protection products on birds and mammals - Feedback on a French approach

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Bird and mammal risk assessments for plant protection products are carried out under EU regulation 1107/2009 using the guidance document issued by EFSA: This document outlines the conservative first-tier assessment procedure for a large range of crops/uses dependant scenarios and proposes different options for refinement if needed. Field studies are related to these assessments. In the regulatory frame, these studies are designed to detect non-intentional effects of the plant protection product in fields of a particular crop, for a particular use and on focal species of birds and mammals. For some plant protection products, post-authorisation monitoring data may be additionally useful to assist in the regulatory process. In this case, the most important aspects are: the knowledge of the structure of the populations of the different species involved. Learning from accidents has been proved to lead to quick reaction and adaptations of regulatory processes, e.g. following the poisoning incidents after drift of honeybee poisoning incidents in Germany, pesticide residues detected and conclusions on the causes for reported incidents will be presented.

Data on the numbers of honeybee poisoning incidents in Germany, pesticide residues detected and conclusions on the causes for reported incidents will be presented.

**TH 254**

Review of the causes of reported honeybee pesticide poisoning incidents in Germany

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Incident reporting is an important monitoring tool to receive information on the reliability of risk assessment of pesticides for bees and the effectiveness of risk mitigation measures. The decrease in bee losses has lead to a proposal and adaptations of regulatory processes, e.g. following the poisoning incidents after drift of insecticidal dusts leading to contamination of flowering bee attractive plants. Even if a strong and sudden damage to colonies is observed, the reason for the bee incident may not be clear at the first view and the extent to which it may be due to specific pesticide application is unknown. Since for several decades an analysis of bee poisoning incidents has been conducted in Germany. A system for analysis and reporting of bee poisoning incidents possibly linked to pesticide applications is well established. Biological analyses, e.g. inspection of bee diseases and, if appropriate, chemical analyses of bee, plant and other samples are conducted by the authorities to investigate the cause-effect relationship between an agricultural treatment and the incident. For the interpretation of data on bee poisoning incidents, an evaluation of the underlying cause of the incident data. Data on the numbers of honeybee poisoning incidents in Germany, pesticide residues detected and conclusions on the causes for reported incidents will be presented.

**TH 255**

Year on year changes in Algerian mouse (Mus spretus) populations inhabiting citrus orchards treated with chlorpyrifos

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The Algerian mouse (Mus spretus) is one of the most abundant rodents across Spain, especially preferring arid areas. Its population densities can range from 3 up to >70 individuals per ha, and fluctuate over the seasons due to phases of maximum reproductive activity in April-May and August-September. Here we present population parameters obtained during field studies in 2009 and 2011 (June to September) from local populations in citrus orchards and adjacent habitats (macchia). Population densities over populations of the different species living in citrus orchards and adjacent habitats and by the year 2011 could be that the carrying capacity of the orchards was reached early in 2011 and therefore the typical second reproductive peak in August could not be observed. Carrying capacity is limited on the weather condition and available resources in a specific area. A population reaching the carrying capacity shows changes in population structure since i.e. birth rate slows down or the emigration rate increases. Therefore it is important to know about the population structure for interpretation of possible effects on small mammal populations through pesticide use.

**TH 256**

Population analysis of the insectivorous Sardinian warbler (Sylvia melanocephala) in chlorpyrifos treated citrus orchards in Spain

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The Sardinian warbler is the smallest predominantly insectivorous bird species inhabiting conventionally treated Spanish citrus orchards, where chlorpyrifos is regularly applied. Assess is difficult to evaluate the importance of commercial citrus orchards during Sardinian warblers’ reproduction period, their seasonal occurrence, site fidelity and age structure was analysed. For this year periodically repeated standardized mist-netting was conducted in ten citrus orchards near Valencia from April to August 2011. Sardinian warblers were present during the entire study period inside the study orchards (i.e. for reproduction). In total 712 individuals were trapped. 134 individuals (18.6%) were captured on at least two occasions and 309 Sardinian warblers were ringed and 3 of these birds were recaptured in 2011. The results obtained support the conclusion that chlorpyrifos treated citrus orchards offer
habitat conditions which are obviously appropriate for breeding Sardinian warblers.

RA17P - Multiple stressors in a changing world

TH 258

Regional scale risk assessment using the relative risk model of threats to the surface aquatic ecosystems of the Umvoti River catchment, South Africa

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The Umvoti River is a highly developed, highly utilised, stressed aquatic ecosystem that is often referred to as a ‘working river’. Potential sources of impacts include numerous urban and agricultural activities, informal settlements, agricultural and forestry activities, industries, sand mining activities and recreational activities. Recent assessments show that a wide range of stressors have been identified in the lower portion of the study area in particular. Existing stressors include water quality and quantity alterations, habitat impacts and associated disturbances to wildlife impacts. Stakeholders of the catchment require a management plan that considers nature and location of multiple sources in the catchment that takes ecosystem dynamics into account and provides threat assessment of established endpoints. The aim of this study is to carry out a risk assessment of the Umvoti Catchment to identify and quantify risks to ecosystem components or habitats in accordance with existing stakeholder objectives or endpoints. In this study the relative risk model (RRM) was applied to selected sources, habitats and endpoints of the Umvoti River system to generate risk and validation hypotheses to test for further risk assessments. Various sensitivity and validity tests were applied to the model to validate the risk outcomes. Findings of the RRM showed that while the upper and middle portions of the catchment have low risks of threats impacting on the endpoints, high risks exist in the lower portion of the Umvoti Catchment. Some additional moderate risks were also identified in the extreme eastern and western parts of the catchment. Within the high risk region, sources of stressors include; sand mining activities, sugarcane activities, heavy industries, urban areas and then sugar mill activities. Endpoints are potentially threatened by water quality, water quantity and habitat impacts. Stakeholder points of concern in the Umvoti Estuary, the sustainable provision of ecosystem services for existing users and a safe and clean environment for local communities. Three hypotheses were developed and tested for further validation of the risk assessment. These tests confirmed the spatial projections and nature of threats established in the risk assessment and reaffirmed threats to endpoints. This risk assessment is currently being used to direct environmental management in the area.

TH 259

Application of Bayesian networks for integrating multiple chemical and non-chemical stressors into a risk assessment using the South River, VA as a case study

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The study examined a method of using a Bayesian network as an alternative risk approach to regional risk assessment lends itself to the construction of Bayesian networks (BN) for the calculation of risk. BN use conditional probabilities based on available data, models or expert opinion to construct the conditional probability table. This table describes the interactions between input nodes and the resultant child node. BNs intrinsically incorporates uncertainty into each of the variables and sets specific criteria and structure for the tabulation of risk. The case study we are using to demonstrate the approach is a contaminant point source in the state of Virginia, USA. The study area is approximately 25 km in length and includes the catchments that directly feed the river. From 1929 to the early 1950s, Hg was deposited into the river from a manufacturing source. We are now estimating risks due to Hg and other stressors up and downstream of the manufacturing site. The BNs were constructed using Netica, and the process of sensitivity and validity tests will be explained in this paper. Smallmouth bass, Kingfisher and water quality parameters are the endpoints examined to date using the BN approach. An interesting result is the interaction between water temperature and contaminant loading in generating an increase in risk far downstream of the manufacturing site. Another feature of the Bayesian approach is that it allows the calculation of restoration strategies and specific management and engineering options. The desired level of risk and the model calculates the required values of the input nodes. BNs are a powerful tool for understanding the impacts of multiple disparate stressors over regional landscapes.

TH 260

Towards a conceptual model of the impact zone ecology in rivers

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Discharge of untreated domestic wastewater into rivers can result in high concentrations of unionised ammonia (NH3), nitrite (NO2) and biochemical oxygen demand (BOD) as well as chemicals used in home and personal care products (HPCPs). In such scenarios, where aquatic communities are severely impacted by conventional waste water constituents (sanitary determinants), it may not be appropriate to conduct conventional risk assessments on HPCP chemicals. An alternative risk assessment model based on the ‘impact zone concept’ has been proposed for these direct discharge conditions. The impact zone, in this case, is the river reach downstream of a direct discharge in which water quality parameters and biological determinants are higher than their predicted no effect thresholds, resulting in potential impacts on community structure and function. Risk assessment i.e. estimation of PEC (Predicted Environmental Concentration): PNEC (Predicted No Effect Concentration) ratio, of HPCP chemicals is conducted at the end of the impact zone. Here, we present a conceptual modelling framework that describes how the ecosystem community composition may change through the impact zone and predict how this could be used to assess the potential impacts of chemicals in HPCPs under direct discharge conditions. A key assumption is that different taxa or biological traits have differing sensitivities to various chemical stressors, which will alter the in-stream community along the stress gradient. At a basic level this can be predicted using species sensitivity distributions, but at higher tiers ecological considerations (e.g. indirect effects) are required.

TH 261

Effects of toxic and non-toxic stressors on fish community in the fish community in the river Rhine

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The Rhine is a long river which has been intensively studied and much effort has been put in the ecological assessment of the fish community in the river. The Rhine has been intensively studied and much effort has been put in the ecological assessment of the fish community in the river. The Rhine has been intensively studied and much effort has been put in the ecological assessment of the fish community in the river. The Rhine has been intensively studied and much effort has been put in the ecological assessment of the fish community in the river. The Rhine has been intensively studied and much effort has been put in the ecological assessment of the fish community in the river.

The present study describes the effects of toxic and non-toxic stressors on fish community in the river Rhine using monitoring data collected since the 1970s and species sensitivity distributions (SSDs). This statistical approach enables us to quantitatively compare concentration related effects (including contaminants, metals, dissolved oxygen, and salinity) with effects related to habitat alterations (like pollution, water quality alteration, pollution, water quality alteration, pollution, water quality alteration, pollution, water quality alteration, pollution, water quality alteration). Therefore, in the present study, the effects of multiple stressors on fish community consisting of native and invasive species are analyzed. The effects of low dissolved oxygen, temperature, stream velocity, and exposure to pesticides and heavy metals are studied with the aim to: (1) identify the difference in tolerance to toxic and non-toxic stressors between native and invasive fish species, and (2) rank these stressors according to the potential risk they have on the overall fish community as well as on the native and exotic component separately, and (3) determine possible changes over time in the sensitivity of the fish community and the relative importance of different stressors in the river Rhine.

The implications of our results for risk management of the Rhine as well as management of exotic fish invaders will be discussed.

TH 262

Multiple stressors in aquatic environments may profile biomarker baseline responses: a case study in Dreissena polymorpha at the population scale

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Non photochemical quenching (NPQ) were studied in control and exposed algal cultures. The experiment followed a factorial design with 2 factors (water temperature and toxicant concentration) and their interaction. A previous experiment determined that the case of diuron, where NPQ mechanisms were inhibited after 12 hours of exposure, suggesting damage in the pigments where the NPQ takes place. Immediately after addition, which indicates a very specific toxic action of this compound to algae (inhibition of photosynthesis). Propanolol and fluoxetine toxicity effects on the algal toxicity of the three compounds differed considerably. Diuron was the most toxic compound, followed by fluoxetine and propranolol. Effects of diuron occurred by a higher mortality and alterations in biomarkers. Toxicological interactions between stressors were also found. These results suggest that the ecological role of E. desmaresti modified (and 4 relatively uncontaminated (relatively unmodified) estuaries. We find that shifts in bacterial communities correlate strongly with changing contaminant concentrations. Sediment contamination was found to select for groups from the orders Rhodobacteriales, Oceanospirillales and Deinocobacteriales, while orders Chromatiales, Cyanobacteria and Rhizobiales experienced declines. It is likely that changes in bacterial community composition as a result of sediment contamination have implications for the functioning of estuarine ecosystems.

Temperature-dependent toxicity: is an assessment factor of 10 appropriate for ecological risk assessment? Combined effects of natural (eucalypt leachates) and anthropogenic (copper) stressors on Echinogammarus meridionalis: implications for ecosystem functioning in a global warming climate scenario.

The increase of temperature reduced the toxic effect of the herbicide diuron and propanolol at the beginning of the exposure as well as at the final time. In the case of diuron, the increase in temperature from 10ºC to 20ºC was found to significantly increase the toxicity of both copper and eucalypt leachates. In the mixtures bioassays, the toxicity of the copper to the same species at a different temperature (T) is expressed as a relative TU value (RTU) which is equal to LC50/TC50. A smaller RTU indicates a higher toxicity. For copper, we detected an average decrease of 0.139 TU/ºC increase in temperature and a decrease of 0.076 TU/ºC decrease in temperature. The results imply that an assessment factor of 10 will cover up to 6.1°C increase and 11.8°C decrease in temperature for copper, translating into a maximum protective range of 17.9°C temperature variation. Global surface freshwater temperature was found to have an average variation of 11.7±1.4°C. Thus, applications of a factor of 10 in this case might be inadequate to protect freshwater organisms. In this presentation, we will present more results on other chemicals before drawing a solid conclusion.

Evaluation of the combined action of natural stressors and chemical pollutants in algae. Assessment of functional, structural and metabolism alteration

Combined effects of natural (eucalypt leachates) and anthropogenic (copper) stressors on Echinogammarus meridionalis: implications for ecosystem functioning in a global warming climate scenario.

Global climate changes may have considerable negative impacts on hypersaline environments of the South of Europe which are also facing other pressures such as chemical
Acute versus chronic temperature dependence of cadmium toxicity in zebrafish
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Toxicity studies are mainly performed in standardized conditions, most often at the optimal rearing temperature of the test organism. However, environmental temperature has a significant effect on the toxicity, as for some test organisms, toxicity is generally increased at higher temperatures. This study aimed at investigating the temperature effect on cadmium accumulation and toxicity. Adult wild-type zebrafish were acclimated to exposure temperatures for one month prior to experiments. Fish were exposed to nominal concentrations of 0, 38, 52, 77, 115, 284 or 435 µM cadmium at 18, 26, 30 or 34°C for 96 h. After exposure, zebrafish were transferred to temperature-controlled water baths and checked for mortality for 96 h in an acute experiment. In a chronic experiment zebrafish were exposed to 0 or 5 µM cadmium for 28 days at 12, 18, 26 or 34°C. Mortality was observed and the cadmium total body burden was measured. The temperature dependence of cadmium toxicity differed substantially between the acute and the chronic scenario. The pattern of acute cadmium toxicity was concentration-dependent. LC₅₀ values were lower at the intermediate temperature (26°C LC₅₀: 102.4 µM) when compared to 18 and 34°C (respective LC₅₀: 200 and 601 µM). The chronic toxicity was concentration-stable and lower than those of acute toxicity (18°C LC₅₀: 18.2 µM, 26°C LC₅₀: 35.4 µM, 34°C LC₅₀: 39.3 µM). For 26°C, exactly in the result of the opposite order of toxicity at low exposure concentrations: 34°C > 30°C > 18°C > 26°C. In the chronic scenario cadmium caused mortality to increase with increasing temperature and the observed differences were much more profound (57% mortality at 34°C vs. 2% mortality at 12 and 18°C) when compared to the acute scenario. Furthermore, in the chronic scenario increasing cadmium accumulation with increasing temperature played an important part in increasing toxicity, while in the acute scenario other physiological processes weakened this relationship. Apparently in an acute highly toxic exposure scenario prior acclimation to an altered temperature protected zebrafish against future stress, while in a chronic scenario the continuous heat stress eventually aggravated cadmium toxicity. More scientific background is necessary to include the temperature effect on chemical toxicity in regulation. Standardized tests are mostly performed at optimum temperature whereas temperatures in the field are often lower. Based on the present results, both in a peak exposure scenario and in a chronic low exposure scenario this could result in overestimation of toxicity.
pollutants in the field. The aim of this study is to compare the toxicity of single exposure of E. andrei to carbayl and the combined exposure with different air temperature regimes (8°C, 12°C, 16°C, 20°C, 22°C, 24°C and 28°C), using survival and biomass loss as endpoints. For this standard acute tests were performed, and adaptations for temperature exposure carried out. To evaluate the joint effect of natural and chemical stressor, observed data was compared with the expected effect predicted by the conceptual model of interactive action (IA) and for synergistic/marginalistic interactions, dose-level and dose-ratio dependency were used. From the result we can changes the toxicity to earthworms depending on the temperature regimes, which provide more realistic information for Risk Assessment procedures.

TH 273

Exposure of Folsomia candida to carbayl and natural stressors: effects on reproduction and genotoxicity
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Terrestrial organisms can be exposed to a great variety of stressors, such as contaminants and/or other physical or biological stressors that affect their life - and genetic traits. Organisms can experience a large range of environmental changes such as temperature changes, drought and flood conditions or even UV radiation increments. The aim of this work is the study of effects of carbayl to a soil-dwelling collembolan Folsomia candida at different abiotic conditions, evaluating their reproductive effort and genotoxicity. Despite the expected exposure to different natural stressors through the combination of moisture and UV radiation. The combined effects were compared to carbayl exposures under standardized conditions: 20°C, 60% water holding capacity. We observed that carbayl induces changes on the survival and offspring production. DNA damage was also detected using the comet assay. Results from the combined exposure showed that fluctuations on environmental conditions such as temperature, soil moisture or radiation can induce changes on chemical toxicity.

TH 274

The effects of copper and temperature on interspecific interaction between two collemabol species, Folsomia candida and Sinella curviseta
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The effect of copper contamination on interactions between two species of springtails (Collembola) at different temperatures (10 and 20°C) has been examined. The instantaneous population growth rates of Folsomia candida and Sinella curviseta have been compared between monocultures and two-species cultures, influenced by different abiotic factors. Temperature proved to have a more significant impact on interactions between the species than copper contamination. However, the interspecific interactions and to generate data that supports the 40%component of a Biotic Lignoid Model (BLM) for metal mixtures. 96-h acute toxicity tests were conducted with individual and mixtures of Cu and Zn, Cu and Ni, Zn and Ni, and larval P. Promelas using moderately hard water. For individual metal exposure, the 96-h LC50s for fathead minnows and Cu and Ni were 207, 873, and 1750 µg/L, respectively. Results of metal mixture exposures indicated that the toxicity of metal mixtures was more than additive (synergistic). When exposed fathead minnows to each Cu at a concentration of 50 µg/L or to Zn at a concentration of 100 µg/L, no significant mortality was observed. However, 27% mortality were produced when exposed the fathead minnows to a mixture of 50 µg/L Cu and 100 µg/L Zn. When increased exposure concentrations 2 times (100 µg/L Cu and 200 µg/L Zn), mortality (23%) produced by individual Cu and Zn exposures was less than the total mortality (60%) produced by Cu and Zn mixture exposure. Similarly, mortality (97%) produced by a mixture of 200 µg/L Cu and 400 µg/L Zn was greater than the total mortality (40%) produced by individual Cu and Zn. Similar results were observed for Ni exposures. At Ni concentrations of ≤ 1000 µg/L, no mortality was observed. However, when exposed fathead minnows to a mixture of 50 µg/L Cu and 500 µg/L Ni, 30% mortality were produced. At 100 µg/L Cu and 1000 µg/L Ni, mortality (76%) produced by mixture exposure greater than the total mortality produced by individual exposures. For Ni and Zn mixture, at 400 µg/L Zn and 1500 µg/L Ni, 67% mortality were produced by the mixture while total mortality produced by individual Zn and Ni was less than 33%. Using toxic unit (TU) concept, the joint toxicity of Cu and Zn, Cu and Ni, and Ni that produced 50% mortality was 0.567, 0.6219, and 1.154 TU, respectively. Results of the present study are useful for the development of a BLM for metal mixtures.

TH 275

Combined effects of chlorpyrifos, mancozeb and temperature on the survival and feeding parameters of Porcellionides pruinosus
C.G. Hodson, A.G. Smith, S. Loureiro

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The aim of this work is study the effects of carbaryl to a soil-dwelling collembolan Folsomia candida at different abiotic conditions, evaluating their reproductive effort and genotoxicity. Despite the expected exposure to different natural stressors through the combination of moisture and UV radiation. The combined effects were compared to carbayl exposures under standardized conditions: 20°C, 60% water holding capacity. We observed that carbayl induces changes on the survival and offspring production. DNA damage was also detected using the comet assay. Results from the combined exposure showed that fluctuations on environmental conditions such as temperature, soil moisture or radiation can induce changes on chemical toxicity.

TH 276

Toxicity of copper, zinc and nickel mixtures to fathead minnows (Pimephales promelas): implications for Biotic Lignoid Model for metal mixtures
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Research on metal toxicity to aquatic organisms has been conducted extensively. However, most research was conducted with individual metals (e.g., Cu, Zn, Ni). This is not always relevant because metals usually present in the natural environment as a mixture of multiple metals. Data on the effects of Cu, Zn, and Ni mixtures to fathead minnows are lacking in the literature. The goals of this research are to characterize the acute toxicity of Cu, Zn, and Ni mixtures to fathead minnows (Pimephales promelas) and to generate data that supports the 40%component of a Biotic Lignoid Model (BLM) for metal mixtures. 96-h acute toxicity tests were conducted with individual and mixtures of Cu and Zn, Cu and Ni, Zn and Ni, and larval P. Promelas using moderately hard water. For individual metal exposure, the 96-h LC50s for fathead minnows and Cu and Ni were 207, 873, and 1750 µg/L, respectively. Results of metal mixture exposures indicated that the toxicity of metal mixtures was more than additive (synergistic). When exposed fathead minnows to each Cu at a concentration of 50 µg/L or to Zn at a concentration of 100 µg/L, no significant mortality was observed. However, 27% mortality were produced when exposed the fathead minnows to a mixture of 50 µg/L Cu and 100 µg/L Zn. When increased exposure concentrations 2 times (100 µg/L Cu and 200 µg/L Zn), mortality (23%) produced by individual Cu and Zn exposures was less than the total mortality (60%) produced by Cu and Zn mixture exposure. Similarly, mortality (97%) produced by a mixture of 200 µg/L Cu and 400 µg/L Zn was greater than the total mortality (40%) produced by individual Cu and Zn. Similar results were observed for Ni exposures. At Ni concentrations of ≤ 1000 µg/L, no mortality was observed. However, when exposed fathead minnows to a mixture of 50 µg/L Cu and 500 µg/L Ni, 30% mortality were produced. At 100 µg/L Cu and 1000 µg/L Ni, mortality (76%) produced by mixture exposure greater than the total mortality produced by individual exposures. For Ni and Zn mixture, at 400 µg/L Zn and 1500 µg/L Ni, 67% mortality were produced by the mixture while total mortality produced by individual Zn and Ni was less than 33%. Using toxic unit (TU) concept, the joint toxicity of Cu and Zn, Cu and Ni, and Ni that produced 50% mortality was 0.567, 0.6219, and 1.154 TU, respectively. Results of the present study are useful for the development of a BLM for metal mixtures.

TH 277

Health of and contaminants in fishes from the Slave/Athabasca River System, Canada, relative to oilsands extraction activities

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The potential environmental impacts of oil extraction activities in the northeastern region of Alberta Canada have recently received significant attention in both the scientific and general media. Chief among the global concerns are the fact that the open pit mining and oil extraction and conversion processes release a variety of environmental contaminants into the aquatic systems. In particular, the water bodies that are affected include the Athabasca River and its tributaries. The potential for these contaminants to accumulate in and disrupt aquatic wildlife are of particular concern to First Nations consumers of ‘wild foods’. Consumption of fish and wildlife from the region are of cultural as well as nutritional and economic significance to First Nations communities. An apparent increase in the occurrence of fishes with apparent deformities and lesions has been reported by local communities as remote from the oilsands operations as the lower Slave River. The aim of this study is to investigate the exposure of fish populations in the Athabasca and Slave rivers to environmental contaminants arising from oil sands activities. In addition to investigating the general health condition of the collected fish and we consider contaminant concentrations in their tissues from both fish and human health perspectives.

TH 278

Influence of environmental factors on the response of Daphnia magna population to pesticide applications
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Most of the risk of pesticides has been performed using standard toxicity tests on Daphnia magna cultured individually. However in the environment organisms experience abiotic and biotic interactions like intersecptic and intraspecific competition that are likely to be of crucial importance to obtain a response to toxicants’ activity. Moreover, input toward surface waters from agrochemicals typically occurs in pulses or in repeated exposures due to agricultural runoff and spray drift. However, the literature on the physiological and metabolic responses of populations to fluctuating and repeated pollutant exposure is very limited. In our multispecies test system we investigated how interspecific competition alters the response of Daphnia magna to pulses of pesticide. Daphnia magna were cultured in two sets of test in which we refer to them “with” and “without” competition showed an acute sensitivity to the pesticide only at the highest concentration tested. Culex pipiens larvae were insensitive at all concentrations. Multiple contaminations did not increase the sensitivity of Daphnia magna to Pirimicarb. Populations in the setup “without” competition were always able to recover within the 28 days between two pulses. However in the “with competition” system we observed no recovery or extinction. Moreover we investigated the interaction between the two species: at all concentrations of Pirimicarb and in the control, the abundance of daphnia was negatively affected by

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the biomass of Culex larvae.

Our experiments revealed that an interspecific interaction can negatively affect the response to pesticide applications of one of the most common test organism in ecotoxicology. We conclude that biotic interactions like competition must be considered when conducting an environmental risk assessment of toxicants.

TH 279
Predicting metal and metalloid partitioning in soils using infrared spectroscopic analysis
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Solid-solution partitioning of a metal (the K value) is a key property that determines its environmental fate in both terrestrial and aquatic systems. The K value can be used to predict exposure pathways and key factors, but it is likely to be the same once a metal is released into the environment. The aim of this study was to derive partial least-squares (PLS) regression calibration models from mid-infrared (MIR) spectroscopic analysis plus soil pH data of soils and experimental derived K values to predict the solid-solution partitioning (log K) of 10 selected elements in soils. The study focussed on Balb/c 3T3 clone A31-1-1 cells. When this established cell line is exposed to carcinogens, cells lose the contact-inhibition property and produce soft-tissue tumours, which are influenced by the outcome of the QS-derivation as compared to the PPP-authorisation. For 65% of the compounds additional acute data were found, additional data on chronic endpoints were not available in the two frameworks, with respect to the treatment of data and the choice of safety factors. During the past years, RIVM has derived water QS for a large number of active substances, for which the chemical parameters of water and thus to improve the development of organisms and the repeatability of the bioassay.

TH 280
Levels and trends of PCBs and PBDEs in fish (Rutilus rutilus and Cyprinus carpio) and sediment from South Kazakhstan

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EPHE - UMR SISYPHE, Paris, France

RA21P - Standard vs non-standard methods for hazard and risk assessment

TH 281
Evaluation of carcinogenic potential of perfluorinated compounds in vitro and in silico alternative approaches

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Perfluorinated compounds (PFCs) are a family of fluorine-containing chemicals used in different applications to make materials oil and water resistant. Regarding human exposure, PFCs are used in wide array of consumer products and food packaging as surfactants. PFCs persist in the environment due to the strength of the carbon-fluorine bond which prevents degradation by natural processes. Because of their persistence, toxicity and widespread occurrence in the blood of humans and wildlife, they are considered as emerging environmental pollutants. Toxic effects of PFCs in laboratory animals includes a range of pathological changes, such as liver and kidney damage and reproductive dysfunctions. Despite the global attention of scientific community, the toxicity and the mechanisms of action of PFCs are still partially unknown: only limited data are reported about the ability of these compounds to work as promoters in carcinogenic process. The aim of this work is to provide an evaluation of the carcinogenicity of these compounds using alternative methods including in vitro methods and in vitro approach. Regarding in silico approach, a selection of dozen software from the list of EU project ANTRAES were used to qualitatively evaluate the carcinogenic potential of PFCs. Freely available (i.e. VEGA, Toxtree, Lazar) and commercial software were used and compared in order to assess their reliability. In addition to the classical QSAR evaluation, some compounds were tested using the cell transformation assay (CTA). In vitro cell-transformation systems have been developed to model the in vivo carcinogenesis process and they are regarded as the only possible in vitro alternative to animal testing for the screening of potential genotoxic and non genotoxic carcinogens. Selected compounds were assessed using the CTA based on human-derived cell line A31-1-1, cells that this established cell line is exposed to carcinogens, cells loose the contact-inhibition, cells loose the contact-inhibition property and produce soft-tissue tumours, which are influenced by the outcome of the QS-derivation as compared to the PPP-authorisation. For 65% of the compounds additional acute data were found, additional data on chronic endpoints were not available in the two frameworks, with respect to the treatment of data and the choice of safety factors. During the past years, RIVM has derived water QS for a large number of active substances, for which the chemical parameters of water and thus to improve the development of organisms and the repeatability of the bioassay.

TH 282
Differences between dossier-based PNECs and EQSs based on all available literature: the case of Imidacloprid
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TH 283
Derivation of water quality standards for plant protection products - the importance of open literature C.E. Smit 

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The European and national authorisation of plant protection products (PPP) is based on registration dossiers which in most cases only include reports of GLP-studies according to accepted guidelines. For quality standard (QS) derivation according to the methodology of the Water Framework Directive (WFD), all relevant available information should be considered. This means that the dossier data provided for example the European or biocides authorisation process should be used, but access to registration dossiers for QS-derivation is often restricted due to confidentiality claims. Data from the open literature are included in the QS-derivation, provided that the scientific reliability is sufficient. As a result, the datasets for authorisation and for QS-derivation may substantially differ. There are also methodological differences between the two frameworks, with respect to the treatment of data and the choice of safety factors. During the past years, RIVM has derived water QS for a large number of active PPP substances. For these compounds, we investigated whether the open literature resulted in additional endpoints that were not included in the dossier, and how this influenced the outcome of the QS-derivation as compared to the PPP-authorisation. For 6% of the compounds additional acute data were found, additional data on chronic endpoints were available for 40% of the compounds. Acute or chronic endpoints below the most critical dossier value were retrieved from the open literature for 27% and 21% of the compounds, respectively. In 37% of the cases, the chronic QS was lower than the authorisation value, with differences of a factor of 2 to 30. This is due to the lower endpoints from the open literature, but also results from the critical evaluation as to whether the potentially sensitive taxa are represented in the dataset. This applies to insecticides and fungicides in particular, since the standard PPP-dossier does not contain chronic data on insects or fungi. Another difference is that transient effects in lower endpoints from the open literature, but also results from the critical evaluation as to whether the potentially sensitive taxa are represented in the dataset. This applies to insecticides and fungicides in particular, since the standard PPP-dossier does not contain chronic data on insects or fungi. Another difference is that transient effects in lower endpoints from the open literature, but also results from the critical evaluation as to whether the potentially sensitive taxa are represented in the dataset.

TH 284
How to cope with variability in laboratory aquatic gnotobiotic microcosms?
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A protocol for ecotoxicological bioassay in 2-l. laboratory microcosms is being developed since 1997 and applied to the study of various pollutants and scenarios of ecotoxicological risk assessment in the field of urban facilities and transport infrastructures (polycyclic aromatic hydrocarbons, municipal solid wastes incineration bottom ash, which runoff residues, dredged sediments, 3DQTS). Effects are assessed on five different organisms (micro-algae, duckweeds, daphnids, amphipods, chironomids) using classical endpoints such as growth, emergence (chironomids), reproduction (daphnids), survival, with a duration exposure of 3-4 weeks. This bioassay can be considered at first glance as a multi-species test, which provides ecotoxicity data for each species present in the microcosm, however it is actually more since it takes into account interactions between test media (e.g. N and P consumption by primary producers, bioturbation of sediment by chironomus larvae, 3DQTS) and interactions between populations (e.g. daphnid grazing of micro-algae, competition for nutrients between micro-algae and duckweeds, 3DQTS). This type of approach is generally considered as valuable since it states between single-species tests, which lack ecological relevance and predictivity, and mesocosm assays, which display drawbacks due to their complexity (high variability). However, even in gnotobiotic systems where the main components are known, variability still exists to some extent and limits the statistical power of the test. In this communication we will illustrate what variability can be in microcosm assays and show improvements brought to the protocol in order to reduce this variability: breedings and cultures of test organisms, microcosm setup, improvement of organisms fitness in control conditions, monitoring and sampling. The best results were obtained when dynamising the microcosm (through renewal of water column), which allows to stabilise physico-chemical parameters of water and thus to improve the development of organisms and the repeatability of the bioassay.

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Detoxification of Daphnia magna of 4 pharmaceuticals and 7 surfactants by activated sludge
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Pharmaceuticals are bioactive compounds generally resistant to biodegradation, which can make them problematic when they are released into nature. The use pattern for pharmaceuticals means that they are discharged into water via sewage treatment plants. Also surfactants are discharged through sewage treatment plants, primarily due to their use in detergents and shampoos and other cleaners. In this study the acute toxicity to Daphnia magna of 4 pharmaceuticals (ciprofloxacin, ibuprofen, paracetamol and zinc pyrithione) and 7 surfactants (C8 alkyl glycol, C6 alkyl glycol, sodium caprylylsulfopropionate, tallow-trimethyl-ammonium chloride, potassium decylyphosphate, propylene glycol ether sulfate and alkylolethanolamide) was determined. The acute LC50s for the pharmaceuticals ranged from 2 µg L-1 to 136 µg L-1. The surfactants showed clear differences in toxicity, with the alkylolethanolamide ethoxylate being the least toxic (0.4 µg L-1) and the alkylolethanolamide being the most toxic (136 µg L-1). These results show that there are large differences in toxicity among pharmaceuticals entering sewage treatment plants, and also that the detoxification of these compounds can vary significantly. Further studies are needed to determine the degradation pathways and detoxification mechanisms of these compounds in order to better understand their environmental impact.
Ecotoxicological assessment of chemical substances is mainly based on standard bioassays, most often single-species tests covering the range of acute and chronic toxicity. In our laboratory we have been developing since 1997 a protocol of ecotoxicological bioassay in 2-L laboratory microcosms and applied it to the study of various pollutants and their effects on aquatic organisms. The first step in the methodical risk assessment is the selection of the right organisms, based on the field of urban facilities and transport infrastructure. Effects are assessed on five different organisms (microalgae, duckweeds, daphnids, amphipods, chironomids) at seven endpoints as established in the standard methods, survival, an exposure duration of 3-4 weeks. The protocol has mainly been used as a static assay, where water was not renewed for the duration of test. A flow-through microcosm assay (dynamic assay) was developed to improve conditions inside the microcosms, resulting in stabilization of physico-chemical parameters, increase of organisms fitness and reduction of variability. Since toxicity is closely linked to the conditions of bioassay, which influence the chemical speciation of the substance and the dynamic of exposure, we aimed to compare informations produced by standard produced single-species tests, multi-species tests with an aqueous phase only, and microcosm bioassays (static and dynamic) on five different species used in the test. Cadmium was chosen as cadmium is toxicant and introduced only at the start of test in single- and multi-specific static assays, and continuously in the dynamic microcosm assay. Cadmium concentrations were monitored in several bioassays, especially in the presence of a sediment phase, due to expected sorption of Cd. Effects on the same organisms in various conditions (duration of test, type of medium, presence or absence of sediment, renewal of water or not) were compared and discussed based on the results obtained. The presence of sediments, leading to decline of water cadmium concentrations and to a multiple exposure of some organisms, complicates the establishment of a link between cadmium concentration and effects, and thus the comparison of simple single-species tests and microcosm assays. However, these assays give a better insight of the fate and effects of cadmium in aquatic environment. Compared to the static microcosm test, the dynamic microcosm test, where cadmium concentrations are kept in the aqueous phase, provides less variable data and is more sensitive.

TH 291

Quality evaluation of existing critical body residue data for baseline neutral narcosis
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The Environmental Risk Effects Database (ERED) developed by the U.S. Army Corps of Engineers and the U.S. Environmental Protection Agency is the most extensive environmental CBR compilation available. ERED was employed for a data quality evaluation of available CBR-effect relationships for baseline neutral narcosis. Evaluation began with an ERED subset consisting of 7574 records for 318 organic chemicals from 651 references for fish, invertebrates, and algae. The first quality screening used 14 different criteria, identifying repeated experiments, lack of appropriate control experiments, invalid data evaluation criteria, and evaluating the number of subspecies/subspecies used in the tests. The data for each chemical were then broken down into 37 chemical subgroups, based on known structural similarities to determine the quality of these data. The remaining 2099 records were then used to determine whether the data for a chemical were high quality or low quality data. The high quality data for each chemical were then used to compare the differences in results obtained using different test methods to determine what test methods are applicable for testing the chemical. The final evaluation was based on these data quality evaluation. This is not to say that these are the only narcotics in the database, or that they always produce toxicity by the baseline narcosis mode of toxic action. Therefore, the objective of this study was to investigate what implications changing test conditions may have on the toxicity of a given compound. The test principle is to expose the algae to a toxicant gradient in the presence of 14C-labelled bicarbonate for 2-3 hours and measure 14C uptake by scintillation counting. Both natural algal communities from Lake Furse (collected at different times of the year), and a laboratory culture of P. subcapitata were used. The algae were exposed to a range of different toxicants such as photosynthesis inhibiting herbicides, acetolactate synthase (ALS) inhibiting herbicides, titanium dioxide and silver nanoparticles, and standard reference compounds such as potassium dichromate and 3,5-dichlorophenol. Preliminary results show no toxicity of ALS inhibiting herbicides neither for the natural algal community nor the laboratory community at the concentrations tested.

TH 294

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TH 295

Validity criteria from standard method: the need for assessor common understanding
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Validity criteria are based on the common understanding of the test principle. In this presentation, we describe lack of common interpretation regarding one of the three validity criteria from the recommended guideline on Freshwater Alga and Cyanobacteria, Growth Inhibition Test. We explore the implications for reliability assessment and the possible ways to increase assessor common understanding of the guideline on Alga toxicity testing.

TH 296

Endpoints and dose-response relationships of low-dose and standardized studies of Bisphenol A
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3Bisphenol A (BPA) is used in large volumes to manufacture polycarbonate plastics and epoxy resins, which are materials used in numerous consumer products. Diffuse emissions of endocrine-disrupting chemicals (EDCs), including BPA, from consumer products to both indoor and outdoor environments have been identified as an emerging issue. In risk assessment and risk management of BPA, the evaluation and interpretation of the low-dose studies (where effects are reported below 50 µg/kg/day) has been extensively debated.

This study aims to provide an overview of endpoints used in the low-dose toxicity studies of BPA, at what levels the studied effects are reported (NOAEL and/or LOAEL), if the dose-response relationships are monotonic or not, and if exposure is prenatal, postnatal or both. These aspects were compared to the studies of the effects of BPA performed according to standardized test methods.

Data were compiled from a comparative review of studies concerning the effects of BPA in the open scientific literature. Endpoints were categorized, e.g. into reproductive...
used for final EQS derivation. Sources were scored for reliability using the Klimisch scores (1=reliable; 2=reliable with restrictions; 3=unreliable; 4=unassignable). Studies with a validity score of 1 or 2 were used for final EQS derivation.

In the REACH dossier, for chronic toxicity in freshwater 52 records are available. Within these records, there is a substantial number of duplicates, and the way the records are presented is not optimized for publication sources such as ‘publication’, ‘study report’, or ‘grey literature’. Thus, public literature is included in the doses, but it may be difficult to determine the original source. Industry has the obligation to conduct literature searches, thereby disclosing all relevant data, but there is no mechanism in REACH that evaluates this aspect. The only formal requirement, however, is at a minimum fulfilling the basic requirement according to the Annexes VII–X. With the Klimisch scores, they cannot be applied in addition to the source limits for EQS derivation. For instance, studies in the dossier for silver which were performed using a slightly shorter or longer exposure time than what is described in the OECD guideline, received a reliability score of 3 (‘unreliable’) while in the EQS derivation they could still be seen as reliable.

Recently, the Klimisch scores in the REACH dossier are divided into key studies and supporting studies. In the silver dossier, there are a number of supporting studies with a reliability of 1 and a number of key studies which refer to ‘grey literature’ without further details. The rationale behind this division is not described in the publicly available part of the dossier. Often, there is no publicly available explanation as to how PNEC values are derived using the available data. This makes a comparison between the PNEC and the EQS difficult.

TH 298

Comparison of methods of application on the toxicity of a test substance to the carabid beetle Pocelus cupreus

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For soil incorporated substances it is important that the method of application used in evaluation of the effects on the soil dwelling non-target species Pocelus cupreus represents a more realistic situation in regard to exposure and bioavailability than the standard sprayed application in which the carabid beetles, food and substrate are contaminated.

Direct spray application of an insecticide product was compared to simulated shank-chisel and drip irrigation applications with the objective of demonstrating that the method of application has a direct influence on the survival, behaviour and predatory activity of adult carabid beetles. For the simulated shank-chisel and drip irrigation applications, the test substance was applied at rates equivalent to a field application rate and volume. Beetles were introduced into the test chambers after application. The comparative spray applications were made at the same rate using a compressed air single lance sprayer calibrated to deliver a spray volume equivalent to 400 Lha, with the beetles introduced into the chambers 2 hours later. Control treatments were applied using the same methods. Post application one Musca domestica fly pupa was introduced into each test chamber to provide a food source.

Survival, abnormalities (affected or moribund beetles) and food consumption were assessed and recorded at intervals for 21 days after application. The results showed that there were significant differences in the survival of the adult beetles between the methods of application. Investigation into the relevant application method of appropriate data.

TH 299

Modification of the OECD 117 Partition coefficient with the HPLC method for high log Kow substances

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Estimation of bioaccumulation potential of an organic substance may be obtained through measurement of its partition coefficient. The current OECD/ACMU method based on HPLC is established up to 6.50, respectively, based on the highest reference substance (DIT) recommended, and extrapolation is expected to lead to high uncertainty. The slow-stir method is not either designed to go higher than log Kow = 8.

However, a low log Kow of 4 or extremely high values >9 for Canada or >10 for the EU are considered strong indicators of low potential for bioaccumulation. BCF is expected to increase with log Kow, increasing up to a maximum at approximately 7.5, but then decreasing thereafter. Substances with very high log Kow (>9) are not expected to bioconcentrate greater than 5000 (for Canada) and with log Kow >10, BCF is expected to be >200, these values being the thresholds for the B criterion in PBTV/PVb assessment under REACH.

Therefore, for test substances beyond the application range of guidelines into force, the limit value does not allow a definitive conclusion on bioaccumulation potential and no method has yet been demonstrated to log Kow >9.

This poster illustrates an attempt to select new reference substances for the HPLC method, with higher log Kow values (7 to 10), in order to obtain adequate result for bioaccumulation assessment. The KowWin database was screened for homologous compounds, with linear increase of their log Kow as a function of carbon number, and with suitable solubility values. A total of 18 candidates between 6.97 and 10.89 was selected, among which 9 of analytical grade are commercially available. However, the method may be subject to limitations in this working range, and suitability of this new set of reference substances for high range Kow determination have to be confirmed by experimental investigations before any update proposal of the guideline.

TH 300

Poorly soluble cosmetic compounds - ecotoxicity and substitution and substitution - (ECOSM)


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In order to meet different product requirements, ‘Personal Care Products’ contain a set of various substances. Each substance is meant to fulfill a specific scope in the final product. The water solubility of poorly soluble substances is characterised by low water solubility (≤ 1 mg/l) and represent important ingredients in many cosmetic products. Due to their large use quantities a reliable assessment of their environmental behaviour and their toxicity to aquatic organisms becomes necessary, e.g. as requested by the European REACH-Regulation. However, at the threshold of the water solubility of such substances, ecotoxicity tests are very difficult to conduct. High lipophilicity and thus extensive adsorption of the substance to surfaces, e.g. to test vessels and organisms, complicates the test performance. The majority of existing standard tests provide inadequate test results that can hardly be used for an appropriate risk assessment. The ‘poorly soluble approach’ is one promising approach to solve this issue. The basic assumption is that the concentration of highly lipophilic and poorly soluble substances and, thus, their exposure to aquatic organisms is very low in the aqueous phase of water bodies. As a result of an extensive analysis of ecotoxicological data, the so-called aquatic exposure threshold of no ecotoxicological concern (ETNCaqu) could be defined for inert substances with a narcotic mode of action. Neither acute nor long-term adverse effects are anticipated on aquatic organisms when concentration of such substances is below ETNCaqu = 1,9 mg/l. For the screening of potential ecotoxicological substances with PBT-properties, the so-called ‘simplified approach’ also seems to be present. Due to its potential relevance for assessing the environmental risks of highly lipophilic substances, the scope of the project ECOSM is to develop the ‘poorly soluble approach’ to a pragmatic and reliable evaluation tool. In this context, existing aquatic ecotoxicological test methods have already been adapted to pass dosing techniques and several lipophilic compounds are being tested to validate the ‘poorly soluble approach’.

TH 301

A divergence of paths? Novel endpoints in hazard and risk assessment

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Apical responses on survival, growth, and reproduction have long been the mainstay of ecotoxicology research, integrating effects at the organism level. Yet, as science progresses, there is increasing pressure to accelerate risk decisions and reduce animal use, biomarker endpoints and in vitro data use in early hazard assessment are taking us down a new path. The challenge is in relating these ‘omic’, cellular, and biochemical markers to adverse effects in a relevant and reproducible manner. Bisphenol A (BPA), an important high production volume compound used in the production of epoxy resins and polycarbonate plastics, is also one of the most studied, particularly for potential endocrine interactions. The database of ecotoxicological results for BPA includes traditional guideline acute and chronic data addressing population level effects of growth, survival, and reproduction, as well as a variety of studies with secondary, often unique sublethal or biomarker-type endpoints, and a growing library of ‘omic’, cellular, and biochemical markers.
and biochemical responses. For example, a number of highly replicated fish-life cycle and multi-generational tests have examined growth, development, VGC, genomic markers, and effects in wildlife species. These tests have been linked to sewage effluent containing the natural oestrogens 17β-oestradiol (E2) and oestrone (E1) as well as the chemical 17α-ethinylestradiol (EE2) originating from human excretion. In contrast, little is known about the presence and effects of these chemicals in Australia, where there is a greater strain on water resources. Oestrogens have been measured in sewage effluents at levels comparable with the UK and there is some evidence of ED effects in fish downstream of sewage treatment works (STW's). It is therefore advisable to determine the potential levels of oestrogens entering Australian rivers and to assess the risk to wild fish populations. This study demonstrates the first use of predictive modelling of oestrogens in Australian STW effluent, creating predicted environmental concentrations (PECs) for a set of STW's in South Australia and comparing them to the UK using a modified version of a model previously for risk assessment in both Europe and Japan. The PEC's were accumulated into an E2 equivalent concentration (EE2) and were corroborated against measured concentrations from one UK and one Australian STW to determine their accuracy. These were then compared with the UK Environment Agency's predicted no-effect concentration (PNEC) of 1ng/L E2. Predictive modelling was shown to provide a good method for first tier assessment of the environmental risk of oestrogens in STW effluent. The model overestimated the measured EE2 by an average factor of 1.4 when corroborated with the more robust UK STW data, however the effluent EEQ PEC's for all STW's were 3-11 times higher than the 1ng/L PNEC. The model demonstrated that although Australian STW's serve a lower population, their EEQ's are not significantly different to the UK's, which is likely to be due to the lower flow through Australian STW's which lowers the dilution factor. The results predict that effluents discharged in both the UK and Australia both contain oestrogens at concentrations exceeding the Environment Agency PNEC, suggesting that without sufficient dilution in rivers there is a risk of ED effects occurring in wild fish.

**RA23P - Wastewater effluent discharges: characterisation and understanding potential risks in receiving waters**

**TH 303**

**Risk assessment of WWTPs water effluents using fuzzy logic**

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This paper presents a new methodology to assess the risk of water effluents based on fuzzy logic, a very well-known theory to treat uncertainty and vagueness, especially in the environmental field where many data are not fully available. The method has been tested using the effluent's pollution data coming from 22 waste-water treatment plants (WWTPs) located in Catalonia (NE Spain). The driving force to develop this study has been the increasing need of prioritizing pollutants appointed by different water regulations such as the Regulation 166/2006 concerning the establishment of a European Pollutant Release and Transfer Register. It aims to establish a Community level register of integrated pollutant release and transfer inventories (PRTR) for particular types of manufacture, processing and production facilities. The model is based on the concept that the discharge of effluent can be viewed as measures of effects that are directly related to assessment endpoints for a sound and robust risk assessment. A case study describing this approach for the evaluation and use of both traditional and non-traditional endpoints will be presented from the extensive aquatic database for BPA.

**TH 304**

**Simplification allows assessment of biodegradation of cationic surfactants in activated sludge reactors (OECD 303)**

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The Continuously-fed Activated Sludge (CAS) test is suitable to predict the removal of organic compounds from wastewater in activated sludge plants. However, a method to distinguish between removal by adsorption and biodegradation is not provided in the Guidelines. Accurate knowledge on the relative role of the different mechanisms in the overall removal of pollutants in activated sludge systems is therefore not available. The main operating parameters i.e. hydraulic retention time (HRT) and sludge retention time (SRT) are strictly maintained during operation of a CAS unit. The following equations were used to calculate the maximum concentration in air of surfactant adsorbed on the sludge and the measured surfactant concentration in the mixed liquid suspended solids which were derived from a mass balance for the CAS unit:

\[
C_{\text{surf in sludge max}} = \frac{SRT}{(SRT + HRT)(C_{\text{surf in influent}} - C_{\text{surf in effluent}})}
\]

Biodegradation was assumed not to occur. This maximum concentration of surfactant absorbed on the sludge and the measured surfactant concentrations in the mixed liquid suspended solids allow calculation of biodegradation percentages. The removal of five cationic surfactants with varying adsorption capacities (decylamine, tetradecylamine, octadecylamine, dioctadecylmethylamine and dioctadecyldimethylammonium chloride) was assessed in CAS tests. The removal from the wastewater ranged from 98.8% (dioctadecylmethylamine) to >99.9% (decylamine). Removal by biodegradation was 69% (dioctadecylmethylammonium salt) to >99.9% (decylamine). These removal percentages were compared with modelling results obtained with SimplesTreat. Simple Treat not only underestimates the potential of biological treatment systems to remove surfactants from wastewater but also the fraction removed by biodegradation. Especially, the biodegradation of surfactants with a high potential to adsorb is not enough appreciated by SimpleTreat. The expression should be included in an update of the test guideline.

**TH 305**

**Predictive modelling of steroid oestrogens in sewage effluent demonstrates the potential for endocrine disruptive effects in wild fish populations in South Australia**

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Endocrine disruption (ED) has been observed in wild fish populations worldwide and is particularly well characterised in the UK, where the presence of oocytes in the males of several species of freshwater fish has been a common finding. In wild roach (Rutilus rutilus) these phenotypes have been linked to sewage effluent containing the natural oestrogens 17β-oestradiol (E2) and oestrone (E1) as well as the chemical 17α-ethinylestradiol (EE2) originating from human excretion. In contrast, little is known about the presence and effects of these chemicals in Australia, where there is a greater strain on water resources. Oestrogens have been measured in sewage effluents at levels comparable with the UK and there is some evidence of ED effects in fish downstream of sewage treatment works (STW's). It is therefore advisable to determine the potential levels of oestrogens entering Australian rivers and to assess the risk to wild fish populations. This study demonstrates the first use of predictive modelling of oestrogens in Australian STW effluent, creating predicted environmental concentrations (PECs) for a set of STW's in South Australia and comparing them to the UK using a modified version of a model previously for risk assessment in both Europe and Japan. The PEC's were accumulated into an E2 equivalent concentration (EE2) and were corroborated against measured concentrations from one UK and one Australian STW to determine their accuracy. These were then compared with the UK Environment Agency's predicted no-effect concentration (PNEC) of 1ng/L E2. Predictive modelling was shown to provide a good method for first tier assessment of the environmental risk of oestrogens in STW effluent. The model overestimated the measured EE2 by an average factor of 1.4 when corroborated with the more robust UK STW data, however the effluent EEQ PEC's for all STW's were 3-11 times higher than the 1ng/L PNEC. The model demonstrated that although Australian STW's serve a lower population, their EEQ's are not significantly different to the UK's, which is likely to be due to the lower flow through Australian STW's which lowers the dilution factor. The results predict that effluents discharged in both the UK and Australia both contain oestrogens at concentrations exceeding the Environment Agency PNEC, suggesting that without sufficient dilution in rivers there is a risk of ED effects occurring in wild fish.

**TH 306**

**Inputs of pollutants by urban wet and dry weather pollution in combined sewer systems: pau urban catchment (CDAPP, France)**

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Urban inputs that include households effluents, drainage water, business effluents (e.g. car washes, dental uses, other enterprises, etc.), atmospheric deposition and traffic-related emissions (e.g. brake lining, rubber, treated or untreated waste from treatments plants or discharge to the aquatic environment, are expected to represent today important sources of pollution to the aquatic environment. Wastewater treatment plants represent a major threat to the aquatic environment since they are collecting a wide variety of sources from both domestic and industrial activities and they are continuously discharging various polluted effluents. Urban inputs which could contaminate the transient source may also contain high levels of pollutants discharged directly in receiving streams by Combined Sewer Overflow (CSO). Implementation of the European Water Framework Directive and its affiliated directives require Member States to improve their understanding of priority pollutants in urban areas and obviously within wastewater systems. We present here an overview of inputs of polluted effluents (urban wet and dry processes in the city of Pau urban area (south west France)) which is about 50 km² with about 150 000 inhabitants. 4 rain gauges and about 40 flow meters were installed in all the study area to monitor the combined sewer network. Effluents were followed during about 10 campaigns including wet and dry periods for Suspended Solids (SS), Chemical Oxygen Demand (COD), Total Nitrogen (TN) and trace metals (Cd, Cu, Pb, Zn, Al, Fe, Cr, As, Mn). Emphasis is given to the characteristic, the partitioning and the fate of oestrogens from wastewater inputs during dry periods and wet periods in the combined sewage system of Pau. Concentrations of pollutants were combined with flow rate to assess fluxes that were directly discharge into receiving stream by CSO or carry on to the Waste Water Treatment Plant (WWT). Following a storm event of about 2 hours we showed that this storm event induces an increase of fluxes by a factor of 1.5 to 3 compared to dry conditions. Half to two thirds of pollutants fluxes were treated by the WWT during this period. The remaining part of the effluent was discharged in the receiving stream the river Gave de Pau. Fluxes of trace metals followed generally the same percentage than SS, suggesting that metal load is mainly associated to SS.

**TH 307**

**Demographic, economic activities and health equipments as potential sources of pharmaceutical compounds in wastewater and performance of wastewater treatment in two**

SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
Presence of pharmaceuticals in wastewater is attested by numerous studies. Although important research work is conducted on wastewater treatment plant (WWTP) effluents and river waters discharged to the sea, very few studies exist on the fate and removal of micropollutants during treatment. The main objective of this study was to assess if pharmaceuticals concentrations in wastewaters could be explained by urban parameters in two urban areas in NW Mediterranean Sea. By its continuous sampling, passive samplers detect most substances passing through the sampler, depending on the chemical properties of the compound and the sampling rate of the sampler. Only 40 km separate both catchments, which have similar population density and distribution. However, catchment A has a higher number of inhabitants, health equipment and economic activities. Both WWTPs use the same technology (membrane bioreactor) to treat urban wastewater, but have different inflows (catchment A 10 times higher than WWTP-B). Influent and effluent streams were monitored during 3 campaigns over 3 days with 24 hours composite samples. Pharmaceuticals and hormones were analyzed using LC-MS-MS. Results are shown for 632 studied compounds: acetaminophen (ACT), diclofenac (DIC), carbamazepine (CBZ), sulfamethoxazole (SMZ), isopropyl (IMP) and ß-estradiol (E2). Fluoroquinolones were more than 3 times higher in WWTP-A for ACT, E2, SMZ and DIC, 80 times higher for CBZ and 1350 times higher for IMP. The differences for CBZ and IMP were not only explained by the cities sizes but also by the presence of more potential sources in catchment A than B. Concentrations of DIC, SMZ and E2 were similar in both WWTPs. CBZ and IMP were respectively 10 and 183 times higher in WWTP-A. In contrast, ACT was 2 times higher in WWTP-B. Those concentrations could be explained by a difference in discharge in the treatment plants (TP) and in the respective concentrations in CBZ and DIC TP. Concentrations of TQs were respectively 6-42% lower in WWTP-B. In contrast, IMP, ACT and E2 showed higher than 75% and 99% in both WWTPs. Therefore, removal efficiencies SMZ was quite stable in WWTP-A (74% to 85%) and greatly varied in WWTP-B (0% to 96%). Results showed the presence of potential pharmaceuticals sources: some were strongly suspected, others needed further investigations. By its continuous sampling, passive samplers detect most substances passing through the sampler, depending on the chemical properties of the compound and the sampling rate of the sampler. Removal efficiencies of organic micropollutants in urban catchments were assessed by passive sampling. Results showed that biochemical-treated textile wastewater effluent mainly contained hydrophilic organic matters: triethylene glycol, tributyl phosphate and phthalates, and hydrophobic organic matters: amino acids and humic substances. And after enhanced coagulation treatment, most of the dissolved organic matter was removed from the bio-chemical treated textile wastewater effluent.
two locations. This was largely caused by a small number of pesticides with very low PNEC values being present at this location. Although not significant, all three wetlands showed a slight decrease in environmental risk from location 1 to 4. In terms of removal of organic micro pollutants, constructed wetlands only show a minor efficiency, and seem to be more efficient in biologically vitalizing the effluent.

TH 313
Assessing the potential of constructed wetlands in reducing environmental impact of STP effluents: the WIEF project
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At some pilot locations in the Netherlands, the effluent of sewage treatment plants (STPs) is lead through a constructed wetland (so called 'waterharmonica') before being discharged to surface waters. This has been shown to be a beneficial practice. The WIEF (Waterharmonica Improving Purification Effectiveness) project studied the potential of such wetlands to reduce the environmental impact of STP effluents on the receiving water, with special emphasis on ecotoxicological aspects. The changes in effluent quality of three STPs was monitored during the passage through constructed wetlands. This monitoring program comprised sampling, chemical, microbiological and biological and gene expression responses of chronically exposed stickleback (Gasterosteus aculeatus). The monitoring was performed between 2008 and 2011 and generated an extensive dataset. In this presentation we will give an overview of how different environmental relevant end points were affected during the passage through the constructed wetlands.

In terms of STP characteristics the STPs were found. A wide range of organic substances were detected in the passive samplers, but in general these were not affected in a structural way by the passage through the wetlands. Occasionally peaks in toxicity were observed, that indicate a potential risk for the receiving surface water. The intensity of these toxicity peaks was reduced after passage through the wetland. No indications were found that the effluents in general affect the reproductive success of the fish. However, some temporal low to medium toxic effects could be verified. The endocrine disruption was less at the end of the wetland where the effluent is about to be discharged on the surface water. This observation is in line with the results from the in-vitro-assays for estrogenic potential, and with the observation that the microbial community in the wetland sediments has a high potential to reduce estrogenic activity. Finally, it was found that densities of pathogens in the effluent were substantially reduced during passage through the wetlands. Constructed wetlands can thus help to improve the environmental quality of STP effluents. Especially by levelling off high peaks in the toxicity and by reducing the density of pathogens.

TH 314
Inventory of emissions of priority hazardous substances in the surface waters in France
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INERIS, Vernouil-en-halatte, France

Introduction
According to Article 5 of the Directive 2008/105/EC on Environmental Quality Standards in the Field of Water Policy, Member States have to establish an inventory of emissions, discharges and losses of all priority substances and pollutants listed in Part A of Annex I to this Directive. This paper aims at describing the French approach used to perform this inventory, and to give indications on the methodology and few definitions:

- the general working scheme of the methodology and few definitions;
- the data needed for the french approach;
- a methodology applied on French river basins;
- the data compilation process.

Methodology guidelines
Methodology definition was guided by the following key ideas:

- A state-of-the-art methodology in agreement with regulatory requirements, and EU guidance.
- A methodology based on specific ecological approach. The methodology is based on efficient discharge data; in their absence some explicit estimation formulas are proposed.
- A single methodology applicable at different spatial scales.
- A methodology that will work despite scarcity of information and data, and uncertainties.
- Functionality as a management tool for designing strategies for the reduction of the impacts of chemical pollution in watersheds.
- A methodology which can easily deal with data from different years. All available information which are representative of a given year to draw a coherent picture of the micro pollutants releases in surface waters during this year must be used.

This approach was also pragmatically handled. It also presided over the construction of the methodology developed in this paper in terms of work efficiency.

Acknowledgement - The author thanks the French National Agency for Water and Aquatic Environments and the french Ministére de l'environnement, du développement durable des transports et du logement for their financial support and the “GT_inventaire” working group for its technical help.

TH 315
Corbicula fluminea: just an invasive species or also a freshwater bioremediator?
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University of Coimbra, Coimbra, Portugal

Much attention has been drawn to the industrial and ecologic impacts of Corbicula fluminea, an invasive freshwater bivalve. This species has also been extensively studied as a potentially toxic metals and organic compounds. Such an attribute lies on the ability of these clams to bioaccumulate chemicals in body tissues together with a fairly large tolerance to the effects resulting from exposure. Additionally, the Asian clam seems to alternate filter-feeding at very high rates with pedal-feeding and deposit feeding, and hence an integrated view concerning different ecosystem compartments can be considered. As to our knowledge no attention has been devoted so far to the effects that these species of the Asian clam may provide if applied to the treatment of contaminated material or even on the nature of the micro pollutants releases in surface waters during this year must be used.

The main aim of the present study was to generate preliminary results on the evaluation of C. fluminea bioremediation potential in the treatment of several effluents with industrial and commercial interest. This study is part of a broader assessment that considers a battery of wastewater effluents such as acid mine drainage, and pulp and paper mill effluents. The toxicity of the initial clam-feeding solution and the post-filtration effluent to non target organisms (the bacteriae Vibrio fischeri, the microalgae Pseudokirchneriella subcapitata and the cladoceran Daphnia sp.) was assessed through laboratorial bioassays. These bioassays were supported by the analytical characterisation of the exposure media before and after the clam filtration. Although the Asian clam is an invasive species and problems may arise from the intentional introduction in the discharge environments, controlled systems that allow us to capitalise on their filtration and bioaccumulation capacities may be assembled. Such a wastewater treatment method could bring huge benefits (e.g. output improvement and costs reduction) in the industrial context.

TH 316
Acute toxicity analysis of urban septic tank sludge
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Federal University of Espirito Santo, Vitória, Brazil

The objective of this work was to evaluate possible toxic effects of sludge samples from urban septic tanks and upflow anaerobic sludge blanket (UASB) from a wastewater treatment plant located in the University of Espirito Santo, in Vitória, Brazil. The method employed was the acute toxicity test Microtox (Azur Environmental) based on the luminescence inhibition of the marine photobacterium Vibrio fischeri. It is a test with many advantages such as ecological relevance, sensitivity, reproducibility, standardization and simple execution. A series of physical-chemical parameters were also analysed based on Standard Methods for Examination of Water and Wastewater 20th (APHA - 1998). Sludge from four septic tanks was used in the study. From each tank four samples were collected, each aliquot representing a distinct depth, classified as: 1. surface liquid 2. superficial sludge; 4. bottom sludge and 5. mixture from all the previous. From the UASB equivalent fractions were also sampled. The tests were executed according to the Microtox User’s Guide Manual (AZUR Environmental, 1996) and results were expressed in EC50 (Effective Concentration), a value that represents the concentration in which 50% of the bacterial luminescence is inhibited. Only in one of the septic tanks, located in a public building toxic effects could be verified in the middle layers (sludge) and liquid waste. In all samples the EC50 was > 1000000 mg/l. In terms of removal of organic micro pollutants, constructed wetlands only show a minor efficiency, and seem to be more efficient in biologically vitalizing the effluent.

TH 317
Comparing chemical analysis with literature studies to identify micropollutants to be treated or upstream source controlled in a catchment of Copenhagen (DK)
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The European Water Framework Directive aims at obtaining good ecological and chemical status of European water bodies by the year 2015 (EU, 2000). The directive also requires that a comprehensive monitoring program be designed and performed in order to assess the status of water bodies in terms of both ecological and chemical quality. The European Water Framework Directive aims at obtaining good ecological and chemical status of European water bodies by the year 2015 (EU, 2000). The directive also requires that a comprehensive monitoring program be designed and performed in order to assess the status of water bodies in terms of both ecological and chemical quality.
Toxicity identification evaluation for wastewater treatment plant working at different industrial and municipal scales

D. Di Marzio1, M.E. Säär2, J.L. Alberdi3, S. Curtesiu3, B. Haggett3

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A toxicity identification evaluation according international consideration was performed taking in consideration the system used for depuration of wastewater. Process control in the WWTPs are a function of the toxic compounds fraction of the river water, which are discharged. The acidic/basic compartments have a concentration of the higher toxicological risk and persistence of the fine particles fraction of the river water, which are discharged. The acidic/basic compartments have a concentration of the higher toxicological risk and persistence of the fine particles fraction of the river water, which are discharged.
In last decade, there has been growing public concern of contamination of water and environment with anthropogenic compounds and their degradation products and possible negative influence to the nature and public health. As a response to this fact, there is an increased interest in more efficient screening techniques of larger number of compounds compared to that traditionally carried out by triple quadrupole mass spectrometry. These substances, (i.e. nonylphenol and ibuprofen) are known as the main exposure route for both kinds of substances. The emission of these chemicals into WWTP originates from consumer usage of products, articles and drugs. The main objective of the project presented in this paper financed by TOTAL and the CONCAWE, is to show the real distance between WEA methodology which is conservative and in situ impact assessment for risk assessment. In this project, the difference between WEA and in situ impact assessment is measured using dynamic outdoor mesocosms. This project has been designed and realized in three successive steps: (1) Preliminary experiments in Laboratory to assess the best solution to store one effluent sample in mesocosms; (2) Optimization of concentration to keep the mixture as close as possible to the observed variations in WWTP; (3) Final experiment in mesocosm with several effluents sampled in different sites and comparison with WEA methodology.

In this presentation the design of the three steps of this project will be presented and discussed.
The “washing solutions” prepared with hand dishwashing detergents were found to be more toxic than those prepared with dishwasher detergents, except for the gel products. Reproduction of *C. dubia* was the most sensitive endpoint compared with the inhibition of the mobility of *D. magna* and the growth of the algae *P. subcapitata*. 

BLMs assume a composition for the DOC in terms of the ratio of humic and fulvic acids. These default DOC characteristics work well for natural waters with generally good humic or fulvic acids. The DOC in effluents will, therefore, almost certainly interact with metals in a different manner to that predicted by BLMs. 

Agreement between predicted and observed no effect concentrations. Sewage effluent contains high concentrations of DOC but a significant fraction of this organic matter is not covered by “European Ecolabel”. These results suggested that the ecotoxicity recorded for ecolabelled products should be weighted regarding to the intrinsic ecotoxicity of the rinse aids which are currently not covered by “European Ecolabel”. 

**TH 330**

Environmental impact of current reserve-antibiotics with increasing incidence of severe infections using modern waste water treatment

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Antibiotic resistance is increasing since 30 years. Antibiotics previously used as reserve antibiotics, e.g. ciprofloxacin, are found in guidelines for treatment of slight infections. Due to uncritical prescription of broad spectrum antibiotics the amount of antibiotics used rises constantly. Many environmental studies proved the existence of different types of antibiotics in hospital sewage, municipal waste water, sewage disposal facilities and surface water or soil. For some substances critical concentrations with effects on algae, bacteria, daphnia and higher life forms were observed.

At the present Riftaxia, Piperacilllinor, Doripenem as well as the current reserve-antibiotics Tigecycline, Linzolide and Daptomycin are not classified as relevant to the environment because of their minor application. Hence environmental data for these substances are missing, as well as future schemes of use. Nowadays most environmental studies are focusing on older frequently prescribed substances. As a result assessment lags behind the modern pharmacotherapy and the achievements of the pharmacology. 

Our objective is to study potential effects on the environment of above antibiotics. Ecotoxicological testing is performed in standardized bios assays using 24- and 96-well microplates for algae and bacterial growth inhibition tests. Ecotoxicological testing with algae growth inhibition test of new antibiotics showed increasing toxicity for Daptomycin and Tigecycline with EC50 values of 14.4 mg/L and 1.76 mg/L. There is no toxic effect for Doripenem and Linzolid at maximum test concentration (EC50 > 1000 mg/L).

Estimating PEC/PNEC relations of these new substances has to take into consideration the increasing number of infections, change in demographics as well as biodiversity, compartment-disturbance tendencies and additional waste-water treatment techniques e.g. ultraviolet light, ozone and membrane filtration.

**TH 331**

Comparative study of the ecotoxicity of dishwasher detergents

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This study aimed at assessing the ecotoxicity of dishwasher detergents. Indeed, despite the high consumption of such products, available information regarding their environmental impact is limited. Thus, twenty-five of the most representative products available on the French market were selected, covering the different dishwasher detergent categories: “multibenefit”tablets, “classical” products (tablets and powder) and “gel” (both “classical” products and “gel” need the use of rinse aid). Our approach was taken into account the ecotoxicity of solutions simulating a wash cycle in a dishwasher in typical use conditions, as recommended by the manufacturer.

The “washing solutions” from selected battery of biosays were relevant and allowed the classification of the different dishwasher detergents. Mostly, it was shown that the reproduction of *C. dubia* was the most sensitive endpoint compared with the inhibition of the mobility of *D. magna* and the growth of the algae *P. subcapitata*. The “washing solutions” prepared with hand dishwashing detergents were found to be more toxic than those prepared with dishwasher detergents, except for the gel products. Regarding the automatic dishwasher products, it was shown that the ecotoxicity of “washing solutions” from the “gel” products was higher than the ecotoxicity recorded for “classical” products and “multibenefit” tablets. This study also revealed that the ecotoxicity of “classical tablets” was, most of the time, higher than the ecotoxicity of “multibenefit” tablets. Additional studies confirmed that the ecotoxicity recorded for “classical” products was mostly due to the addition of rinse aid in the “washing solution”. These results suggested that the ecotoxicity recorded for ecolabelled products should be weighted regarding to the intrinsic ecotoxicity of the rinse aids which are currently not covered by “European Ecolabel”. 

**TH 332**

Characterisation of dissolved organic carbon in effluents and the assessment of its overall benefits in mitigating environmental impacts from metals

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5University of Adelaide, Adelaide, Australia

Dissolved organic carbon (DOC) is known to form complexes with metals which can markedly reduce the free concentration of the metal and mitigate toxicity. Biotic Ligand Models (BLMs) for metals include speciation calculations of DOC with the metal ions, with increasing DOC concentrations usually providing a significant reduction in the availability of the metal. 

BLMs assume a composition for the DOC in terms of the ratio of humic and fulvic acids. These default DOC characteristics work well for natural waters with generally good humic or fulvic acids. The DOC in effluents will, therefore, almost certainly interact with metals in a different manner to that predicted by BLMs. 

As a result assessment lags behind the modern pharmacotherapy and the achievements of the pharmacology. 

Our objective is to study potential effects on the environment of above antibiotics. Ecotoxicological testing is performed in standardized bios assays using 24- and 96-well microplates for algae and bacterial growth inhibition tests. Ecotoxicological testing with algae growth inhibition test of new antibiotics showed increasing toxicity for Daptomycin and Tigecycline with EC50 values of 14.4 mg/L and 1.76 mg/L. There is no toxic effect for Doripenem and Linzolid at maximum test concentration (EC50 > 1000 mg/L).

Estimating PEC/PNEC relations of these new substances has to take into consideration the increasing number of infections, change in demographics as well as biodiversity, compartment-disturbance tendencies and additional waste-water treatment techniques e.g. ultraviolet light, ozone and membrane filtration.

**TH 334**

Integrating sewage treatment plant monitoring and modelling results to prioritise chemicals of concern and assess environmental risks

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American organic chemicals released via sewage treatment plant (STP), prioritising pollutants of concern and assessing their risks for the aquatic ecosystem entail key scientific challenges for regulators. From the EU perspective, such challenges are common to the chemical legislation, (e.g. REACH regulation, Biocidal Product Directive and the water legislation (e.g. Urban Water Treatment and Water Framework Directives). In chemical risk assessment, the exposure of the aquatic ecosystem to organics is calculated via STPs is calculated in two steps: first, local and regional emissions to sewage are estimated from consumer use data; then, the fate in a generic STP is calculated using the multimedia box model SimpleTreat. Default calculations are based on conservative (worst-case) assumptions.

A validated model was carried out for two chemicals of concern in class and triclosan, to evaluate the existing modelling framework applied to regulatory chemical risk assessment in the EU and to explore synergies with the monitoring and risk assessment activities under the Water Framework Directive.

Concentrations in raw sewage were estimated from product usage and chemical inclusion levels. The distribution and elimination in a conventional activated sludge sewage treatment plant with primary and secondary sedimentation was calculated with SimpleTreat 3.11, from the physicochemical properties and the biodegradation rate derived from STP simulation tests (OECD 303A). Probabilistic model simulations were run to account for the uncertainty of model inputs and the variability of STP parameters across the EU.

Calculated STP influent and effluent concentrations were in good agreement with measured concentrations collected from the literature and the probabilistic simulations captured, though not completely, the observed spread in observed values. Such modelling predictions can complement existing monitoring data and fill gaps for data-poor chemicals. The comparison of predicted and observed concentrations facilitates the comparison of data generated and the harmonization of methods applied across regulations.
TH 335

An aerobic biodegradation of PCBs in a grass cut batch reactor

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Due to properties such as high Kₐ values chlorinated compounds including PCBs, dioxins or chlorinated pesticides in vegetation can lead to bioaccumulation in livestock and in the food chain. Hence, the remediation of these compounds in the environment is an important factor for human health. The degradation of the persistent Polychlorinated Biphenyls (PCBs) under anaerobic conditions in sediments and sewage sludge is described in the literature [1,2]. The potential for degradation in an anaerobic grass cut batch reactor has not been performed before and is investigated in this work. Therefore two series of batch experiments in laboratory scale (0.5 L to 1.5 L of volume) were performed under mesophilic conditions.

The first experimental series was performed at low natural, i.e. not spiked concentration level of Elbe riverside grass and sludge samples at 27 °C for 315 days. This first, explorative experiment showed ambiguous results for the degradation of the 6 indicator PCBs (PCB No.: 28, 52, 101, 118, 138, 180). Concentrations of the lower chlorinated PCBs (Nos. 28, 52) were identified as the best candidates to be degraded with higher chlorinated PCBs (Nos. 101, 118, 138, 180) were found to be stable at 520 µg/kg dry weight sewage sludge. For the second experimental series 6 PCBs (No.: 28, 52, 101, 169, 138, 180), a technical mixture of PCBs (Aroclor 1260) and the pesticide Endrin were added in high concentration to the batch experiments (33 µg compound, respectively and 100 µg of Aroclor 1260). In this second experiment different initial weights of grass cut from the riverside was used and a series of sludge was prepared. For determination of degradation rates experimental times for these batches vary between 30 and 120 days. Concentration changes were monitored as GC-ECD. Methane and carbon dioxide concentrations (measured by FTIR) in the gaseous phase of batch experiments were used as an indicator for biological activity. Results from the second batch experiment, concerning PCB-degradation rates and their effects on carbon and methane production kinetics due to different dry masses, are discussed on the poster.

References

TH 336

Removal of nutrients by immobilized microalgal beads in a continuous flow system

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Using immobilized microalgal beads in contaminant removal is an emergent method in recent environmental studies. As is well known, phytoplanktons use nitrate (N) and orthophosphate (P) during the photosynthesis process for production. In this study, a diaoton "Phaeodactylum tricornutum" was immobilized in 3.5 % sodium alginate solution, and introduced into a continuous system to remove nitrate and orthophosphate. Daily samples were taken from the system and analysed by using UV-Vis spectrophotometer (Chebios Optimum-one) to determine the removal efficiency of the nutrients by the algal beads. The cell numbers in the beads were also determined by counting in a Coulter Counter system during the experimental period. The blank beads were also run in the system and subtracted from the total amount of the nutrients to detect the net removal by the algal system. The system was operated as six separate runs with usage of the same algal beads by starvation of three days at each time to increase the removal efficiency of the system. It was observed that the algal beads remove approximately 50% and 40% (average values of net removal by the algae) of the N and P respectively, considering the inflow concentrations of 1000 mg/l of P and 3000 mg/l of N. Dissadvantages of this method are indicated as the release of the microalgae out of the beads during the experiment and disintegration of the algal beads over a long term run.

TH 340

Towards transparent and relevant use of energy use indicators in LCA studies of biofuels

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When performing the life cycle assessment (LCA) of petroleum products, life cycle inventory data are generally global compilations and they are not partitioned into sub-processes, but rather into black boxes. Several products with different characteristics and uses are included, which makes it difficult to allocate the impacts of energy consumption and emissions for each product is a difficult task because many of them are connected. Therefore, energy consumption and emissions are allocated according to the final product distribution. The most allocation methods used for petroleum products are based on the consumption of crude oil and the amount of energy content, according to the individual life cycle stage of the product.

This study compile the results of several studies of diesel production in European and American processing plants in terms of GHG emissions and energy consumption. Furthermore, this study has been completed by recent studies of diesel production in Spanish refineries. Results highlight the broad range of values that depend on variables such as the plant efficiency and the degree of specialization. Spanish refineries values are within the range of average European plants. Conclusions draw attention to the need of real data from refineries in order to obtain more adjusted and accurate results, as well as the possible partial subdivision of the system as a solution, according to ILCD handbook recommendations for allocation procedures.

TH 341

A meta analysis review: ‘Drop-in’ Biofuels - Life Cycle Assessments, substance flow analysis, material flow analysis and relevant information on an advanced biofuel

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The biofuels and related sector is enjoying an unprecedented amount of public and private effort in order to reduce the environmental and technical barriers. One of these very important barriers is the challenge of developing biofuels that are compatible with existing gasoline, jet fuel and diesel infrastructure. The United States Department of Energy has been championing efforts that ensure that the next generation of biofuels will be regarded as “drop-in” biofuels. According to the former Undersecretary of Energy, Dorothyritional, "drop in" biofuels can be defined as fuels produced from various biomass which are compatible with the oil refinery and gas station infrastructure currently available in the United States. According to definitions used in literature review, drop-in biofuels are described by National Advanced Biofuel Consortium of the United States as infrastructure compatible - they can either be used directly or blended with their petroleum-derived counterparts. The European Commission on the other hand defines drop-in biofuels on the basis of fuel quality specifications (standards) developed by the American Society for Testing and Materials. Numerous LCA works have been conducted concerning what is described as drop-in biofuels. Although this topic of drop-in biofuels is relatively new, literatures regarding LCA studies on these topics can be found. A comprehensive literature review based on the definition provided was available for analysis. A comprehensive literature review was conducted on the previous conducted LCA studies in this new field of advanced biofuels. Also, important topics concerning this new chapter in the biofuels revolution were delved into. The results of this meta-analysis review will be presented at the SETAC world congress.

TH 342

How to assess the data quality of LCI studies - a systematic approach

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The field of energy is from biomass broadly growing, increasing the need for reliable data on the environmental burdens inflicted by the use of the different conversion technologies. A huge number of LCI studies on these topics can be found, however, most of these studies are not directly comparable to each other and their results may vary considerably depending on different data origins, time and geographical background, technologies and system boundaries.

Therefore it is often unclear how to decide which study is “best” suited for a given case. The objective of this work is to present a guideline that helps compare different LCI studies. In this guideline a systematic approach on the assessment of literature quality is done. In a first step the current “best practice” for modelling an LCI is summarized. By this a reference for the assessment of the data is defined. This reference will identify a
Our general strategy is to take a more qualitative approach to the problem of divergence or discrepancy in LCA. We think about a web-based toolkit that tries to resolve residues or wastes should be allocated, however a clear definition of these is lacking. Currently, in order to incentivise biofuel production from second generation sources such as second generation biofuels, the current economic model is based on the assumption that the cost of feedstock is lower than that of fossil fuels. However, this is not always the case. There are various factors that influence the cost of feedstock, such as transportation costs, agricultural yields, and government policies. In this way the divergencies will be directly resolved at the level of the Data Access Layer. The (free-to-use) basic system we envision will support simple assessments and will help LCA users to produce more robust and policy-relevant results.

TH 345
Finding LCA research direction with the aid of meta-analysis
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2University of Bath, Bath, United Kingdom

In this paper the authors developed and applied a structured approach, inspired by the meta-analysis concept, to examine literature and identify research thrusts on how to further develop LCA. The procedure consists of four steps: i) definition of the research question, ii) carrying out a literature review concerning more than 280 papers - selected from about 2000 articles according to pre-defined criteria - which resulted in the identification of some 60 main methodological topics; iii) research gap analysis, in which the methodological topics identified in the previous step were compared with the research priorities identified through a users’ survey, and iv) interpretation of results, in which the results of both the previous steps were evaluated and organized into coherent research thrusts. Overall the analysis delivered two main research thrusts: one devoted to increase practicability of LCA, the other to increased model fidelity. The former is aimed at making commodities and economic dependence. On the other hand, the emission of greenhouse gases causes severe effects on the climate with unpredictable impacts on the petroleum-based counterparts. Finding LCA research direction with the aid of meta-analysis

TH 346
Greenhouse gas emissions from wind power: a critical meta-analysis
S.S.B. Chapman
Heriot-Watt University, Edinburgh, United Kingdom

In this paper a range of historic lifecycle studies of greenhouse gas-equivalent emissions for on and offshore wind farms are reviewed to identify a definitive set of the most current, original, and transparent studies. It begins by briefly detailing the separate components of the wind farm life cycle before explaining the methodology of the meta-analysis and exploring the range of estimates. The paper highlights that ranges of emissions estimates are present for wind power. The paper also suggests that meta-analysis combining LCA and input-output analysis, scenario modeling, and new efforts for developing social and economic assessment methods, are only some examples of the developments LCA methodology has been going through. Overall, it may appear that LCA lacks direction on how to further develop. In this way the divergencies will be directly resolved at the level of the Data Access Layer. The (free-to-use) basic system we envision will support simple assessments and will help LCA users to produce more robust and policy-relevant results.

TH 347
Environmental impacts of palm oil biodiesel: A Meta Analysis
Y.M. Manik, A.N.T.H.O Halog
University of Maine, Orono, United States of America

Environmental impacts associated with the life cycle of palm oil biodiesel is reviewed in this study using meta-analytic method. Most studies found palm oil biodiesel would produce positive energy balance with an average net energy ratio of 3.27, which exhibit the strong potential of palm oil biodiesel as renewable fuel. However, it was found that palm oil biodiesel is a net emitter of GHG to the atmosphere. The origin of oil palm plantation is the foremost determinant of GHG emissions. Converting peatland forest to palm oil production generates up to 60 t CO2eq ha-1 yr-1. In contrast, converting degraded land or grassland for plantation can positively offset the system to become a net sequester of 5 CO2eq ha-1 yr-1. Some other cradle-to-grave environmental impacts associated with palm oil biodiesel are acidification, eutrophication, toxicity and biodiversity.

TH 346
Greenhouse gas emissions from wind power: a critical meta-analysis
S.S.B. Chapman
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In this paper a range of historic lifecycle studies of greenhouse gas-equivalent emissions for on and offshore wind farms are reviewed to identify a definitive set of the most current, original, and transparent studies. It begins by briefly detailing the separate components of the wind farm life cycle before explaining the methodology of the meta-analysis and exploring the range of estimates. The paper highlights that ranges of emissions estimates are present for wind power. The paper also suggests that meta-analysis combining LCA and input-output analysis, scenario modeling, and new efforts for developing social and economic assessment methods, are only some examples of the developments LCA methodology has been going through. Overall, it may appear that LCA lacks direction on how to further develop. In this way the divergencies will be directly resolved at the level of the Data Access Layer. The (free-to-use) basic system we envision will support simple assessments and will help LCA users to produce more robust and policy-relevant results.

TH 347
The renewable energy directive and cereal residues
L. Whitaker, C. Mcmannus, P. Hammond
University of Bath, Bath, United Kingdom

The Renewable Energy Directive provides a methodological framework for a streamlined LCA that calculates the greenhouse gas (GHG) balance of biofuels in order to determine whether they reach the required 35% GHG reduction threshold. It describes which sources of emissions should be included and describes how co-products and residues are allocated, however a clear definition of these is lacking. Currently, in order to incentivise biofuel production from second generation sources such as cereal residues and wastes, double credits are to be awarded to these biofuels, and the feedstocks are considered to be available at zero GHG ‘cost’. This presentation will consider if cereal crop residues should be accounted for in GHG reporting for biofuels, as there are potentially sustainability implications with removing residues from agriculture and/or for food markets. The RED does not consider these potential impacts, which questions the ability of streamlined LCAs to account for the sustainability of these biomass resources. This presentation will compare results from streamlined LCAs and a full environmental impact assessment of cereal residue removal and combustion. The results will indicate the importance of integration of direct and indirect LCA impacts in policy analysis.

TH 348
Meta-analysis of LCA studies for bio-based polymers: assessing the environmental performance of polyhydroxyalcanoates (PHA) and polylactide (PLA) in contrast to their petroleum-based counterparts
R. Essel, M. Carsus
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The depletion of non-renewable resources plays an important role in present political debates: On the one hand, the depletion of non-renewable resources results in increasing commodity prices and economic dependence. On the other hand, the emission of greenhouse gases causes severe effects on the climate with unpredictable impacts on the human environment. That is why energy from renewable resources as well as bio-based chemicals and materials have attracted great interest.
Polyhydroxyalkanoates (PHA) and polylactic acid (PLA) are appropriate bio-based substitutes for a variety of petroleum-based products. Life Cycle Assessments (LCAs) according to ISO 14040 and 14044 have been applied to combine the environmental performance of these bio-based polymers in contrast to their petroleum-based counterparts. Numerous cradle-to-gate studies have been carried out to analyse the environmental impacts of the production of polymer resins with a preliminary focus on impact categories climate change and non-renewable energy use. Previous studies indicate a wide range of results and contradictory conclusions due to the consideration of different production processes, methodological choices (e.g. system boundaries, co-product allocation, impact assessment methods) and underlying assumptions. To produce more robust and policy-relevant results than individual LCAs, this study presents a Meta-Analysis approach for a Mega-LCA. The baseline scenario - reference year 2009 - was defined and undertaken to analyse the outcomes of 25 LCA studies. The underlying studies are quantitatively assessed taking into account the impact categories climate change (measured in metric tons of carbon dioxide equivalents) and non-renewable energy use (measured in megajoules). Relative environmental impacts per metric ton of PHA and PLA are compared with corresponding values for the petroleum-based plastics polypropylene (PP), polyethylene (PE), polyethylene terephthalate (PET) and poly styrene (PS). Furthermore, differences between the environmental impacts of bio-based and petroleum-based polymers as well as standard deviations are calculated to derive methodological consistent figures. Results of the Meta-Analysis indicate environmental advantages and disadvantages in both impact categories depending of the selected pair for comparison between bio-based polymers and petroleum-based polymers (PP, PE, PET, PS). Furthermore, the Meta-Analysis reveals that bio-based polymers can help to mitigate climate change and save non-renewable energy.

TH 349
The effect of system boundary and weight of the life cycle factors on the results of a life cycle assessment (LCA) - Electricity generation as example systems
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LCA is a widely used tool for determination of strength and weaknesses regarding ecological aspects of any product or service. It is expected from the scientific-based analysis to deliver robust information, but in fact in literature the results differs significantly for the same product or service. Due to the recognized uncertainty this study addresses the possible deviation of results from literature. The analysis of electric energy production from different energy sources. The potential deviation could arise from the consideration of different system boundaries (SB) and the relevance of emission sources along the life cycle. Both could be mainly responsible for the recognized diversity beside others like allocation, cut-off criteria, actuality, literature, etc.

The electric power generation gives a good instance to analyze the impact on the results of the different SBs. For example: the variation between different plant construction demands is often over 80%, but the overall results don’t necessarily correlate with this deviation. The change of cement demand for construction by 230%, in the case of electricity generation from coal, induces only 1.7-2% difference in GWP of the total system. This is an effect of different proportion of emission sources between the life cycle stages.

The contribution will point out where uncertainties and dependency of the results could arise through the examples of electricity generation from coal(lignite), natural gas, hydro energy, nuclear energy and from wind energy. The whole life cycle of power plant (with fuel upstream), the following system parts and impact categories have been taken into account by understanding the results of LCAs:

- Construction, fuel upstream, transport, production, operation, decommissioning as life cycle phases
- Construction demand variables (basic construction materials: cement, steel, etc.)
- Fuel demand variables (efficiency factors: fuel purity, thermal efficiency, wind yield, transport, etc.)
- Impact assessment methods (GWP as main LCA indicator, etc.)

System boundaries and other variables contribute to the varying results of LCA studies for electric energy production. Data from the literature should only be used from reliable sources and after thorough investigation, if the SBs and information of the used variables is well described. Otherwise the unreflected use of a data source might lead to false results and recommendations.

LC06P - Life cycle management (LCM): Success factors and barriers
TH 350
Using LCA in the preparation of the new waste management plan of Regione Lombardia
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Regione Lombardia has among its tasks the development of management strategies for municipal waste produced in its territory, in collaboration with provincial governments, the control bodies and public and private entities that operate on the collection, recovery and disposal cycle.

In order to pursue sustainability also in this field, Regione Lombardia has decided to consider “environmental performance” as a criterion for supporting future planning decisions, and therefore stressed the need for specific assessments of environmental impacts associated with the planning in the waste field.

Life cycle assessment (LCA) has thus been chosen by Regione Lombardia as a strategic support decision tool in the preparation of its new waste management plan. The goal is to support life cycle planning to address the current strategic situation and to design priorities action levers.

The project (called GERTA: GESTione Rifiuti in Lombardia - Analisi del ciclo di vita) started in November 2010 and will be completed by the end of 2011.

The first phase of the study consisted in the analysis of the present management of municipal waste in Lombardia Region (baseline scenario - reference year 2009)

This has evolved the identification of all the fluxes of materials (both the source-separated fractions and the unsorted residual waste) in terms of quantity, composition and destination, together with the characterisation of the most important treatment plants, in terms of their capacity, energy and materials consumption, emissions in the environment, energy and materials recovery. After that, the LCA methodology was applied to evaluate the energetic and environmental impacts associated with this baseline scenario.

The contribution will point out where uncertainties and dependency of the results could arise through the examples of electricity generation from coal(lignite), natural gas, hydro energy, nuclear energy and from wind energy. The whole life cycle of power plant (with fuel upstream), the following system parts and impact categories have been taken into account by understanding the results of LCAs:

- Construction, fuel upstream, transport, production, operation, decommissioning as life cycle phases
- Construction demand variables (basic construction materials: cement, steel, etc.)
- Fuel demand variables (efficiency factors: fuel purity, thermal efficiency, wind yield, transport, etc.)
- Impact assessment methods (GWP as main LCA indicator, etc.)
- System boundaries and other variables contribute to the varying results of LCA studies for electric energy production. Data from the literature should only be used from reliable sources and after thorough investigation, if the SBs and information of the used variables is well described. Otherwise the unreflected use of a data source might lead to false results and recommendations.

LC05P - Life cycle management (LCM): Success factors and barriers
TH 351
Life Cycle Assessment for end of life computers in Mexico
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The recovery of waste electrical and electronic equipment (WEEE) is an activity that becomes more important every day in Mexico. In 2010 electronic waste generation reached 307,000 tons annually. Of these, 10% is recycled or valorized, el40% remains stored in houses and 50% is sent to final disposal in landfills and open dumps. The aim of this project was to conduct a life cycle analysis using the program Umberto for electronic waste management in Mexico and compared its environmental impacts with those for three proposals for change in the end of life management pattern in order to determine the best option for a national policy. The policy proposals were modeled as follows: 1) Stage with 25% of recovery system , 2) Stage 35% to recovery system, and 3) 0% for disposal in open dumps. As conclusion it was identified to eliminate the use of open dumps for waste disposal in first place, followed by an increase in 35% of the recycling/valorization system.

TH 352
Implementation of an integrated technological-LCA modelling tool within the water industry - a pragmatic contribution to decision-making
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Novel standards in climate change is expected to take into consideration environmental issues. But it still has to deal with operating costs, sanitary risks, technical feasibility and reliability of designed plants. Therefore, the water industry managers need handy tools and practical methodologies that can provide them a complete set of information for projects under study. A good implementation of such decision supporting tool is obviously a condition to its success.

Within the EVALUEAU project, an integrated technological-Life Cycle Assessment modelling tool has been developed with the software Umberto®. The tool is based on an exhaustive library of unit process models. These models are highly detailed and parameterized, thus precisely describing the energy and mass balances depending on a specific project context. Complementary software tools are directly linked to the software PHREOC® which simulates chemical reactions.

Every process unit model generates an engineering design report when placed within a modelling scenario. These reports are spreadsheets containing engineering design data (e.g. pumps power). This information is interesting for the decision stages since it can be made knowing how the process will work on the field. Problematic technological solutions can be avoided whereas convenient technologies will be selected.

A code for sensitivity analysis of the process model parameters is also part of the framework of the tool. The mathematical method followed is the Morris method. It aims at detecting the key parameters of the modelled scenario by qualitatively estimating their influence on any of the results (e.g. one selected environmental impact or the operating costs). This will help the designer understand how the modelling scenario react to a change on one model parameter. Assuming that the modelling scenario is realistic enough, conclusions can be made on the real plant. This key feature of the tool gives the designers good clues about the future plant functioning and indicates which are the priority actions levers.

The role of technology implementation relies on its convenience on the field, obtained by previously developing it closely with its future users. Not only environmental issues are taken into account but the tool give the whole picture of an industrial project.
The company R&D projects were crucial for developing practical guidelines for the industry. Motivation of the whole supply chain of the companies in the beginning of the project is fundamental. Additional workshops for the whole industry gave important feedback and shall facilitate larger implementation of the guidelines.

As many Finnish food companies use carbon footprinting, industry participation is high. The guidelines many Gallup polls. Over the years, have increased the interest of industries to life cycle assessment on company and product level. Growing general knowledge and pressure to act on climate change has

POEMS: Life cycle thinking applied to an immunological product (vaccine) used for boar taint control in male pigs

In 2009, Pfizer Animal Health (USA) started to apply the Life Cycle Assessment (LCA) methodology to some innovative products, with a first case-study on Improvac™, a vaccine. The aim of this abstract is to present a LCM tool designed for the agri-food industry, a POEMS framework with a modular structure resulting from the integration of complementary tools: an Integrated Management System (IMS), a simplified Life Cycle Assessment (LCA) and a suitable Environmental Product Label or Declaration (EPLD) (www.environdec.com); the results of this project were presented for the first time at the 2011 Annual European SETAC Conference in Milan.

A first Environmental Product Declaration or EPD on a veterinary product was published by the International EPD System, a well recognised ISO 14025 Program Operator as an animal welfare issue by many in the public sector.

In 2010, after an initial project which included a consistent data collection from the vaccine production plants as well as from farms and slaughterhouses at global level, the first Environmental Product Declaration or EPD on a veterinary product was published by the International EPD System, a well recognised ISO 14025 Program Operator (www.epd-system.org). The results of this project were presented for the first time at the 2011 Annual European SETAC Conference in Milan.

In late 2011, the sample of farms participating in the global survey was extended for the development of the renewed and updated EPD to be published by the end of January 2012. The new data collection work led to the inclusion of a considerable number of representative farms from other countries worldwide. This analysis provided LCA data to assess possible environmental benefits of the vaccine’s application: the reduction of the carbon footprint is a strong perceived advantage for farms, industries and food sectors and for this reason, it is the major impact category of interest; other LCA relevant impact categories are presented and discussed to provide a comprehensive view of the life-cycle impacts of the product. The calculated carbon footprint for the Improvac pig system demonstrates a reduction vs. the physically castrated pig system, mostly depending on country specific feed production and slurry management practices. Starting from the feed components, a detailed examination was conducted on an agri-food system basis to follow the same holds for stationary rainwater management and best practices in technology.

For this reason, the information collected and analyzed in the LCA process provides an important opportunity for swine producers to understand the magnitude of their

A new LCA tool for measurement at the initiative of the users - a bottom-up approach

The results presented according to the three purposes of the tool: optimisation of the environmental performance of maintenance scenarios, comparison of different scenarios, and benchmarking the scenarios with average Dutch maintenance scenarios. The resulting calculation tools a good example of the effectiveness of this bottom-up approach, aiming to achieve a broad support amongst potential users of this tool.

Improving Life Cycle Management (LCM) tools for the food industry: a framework of Product-Oriented Environmental Management System (POEMS)

The aim of this abstract is to present a LCM tool designed for the agro-food industry, a POEMS framework with a modular structure resulting from the integration of complementary tools: an Integrated Management System (IMS), a simplified Life Cycle Assessment (LCA) and a suitable Environmental Product Label or Declaration (EPLD) (www.dwbiofilters.dk) aiming at improving sand filters for drinking water production by investigating its inherent microbial processes.
The Nestlé Environmental Sustainability team has developed a new water treatment technology called "EcoFiltre", which can effectively remove 99% of pharmaceuticals from wastewater. This technology utilizes a combination of physical and biological processes to achieve high removal rates. The EcoFiltre technology is currently being tested in several pilot plants around the world, including in Switzerland and France. The results show that the technology is effective in removing a wide range of pharmaceuticals, including antibiotics, hormones, and analgesics. The technology has been shown to be cost-effective and can be implemented in existing wastewater treatment plants. The Nestlé team is working with local authorities to implement this technology in a number of cities around the world, with the aim of improving water quality and protecting public health.
The present work was undertaken to investigate the feasibility of cost effective adsorbents: pure fired clay, newly designed fired clay with the polymer addition (5 mass% PEG 600) and bentonite for Zn(II) ion removal from waste printing developer. The adsorption efficiency and distribution coefficient of used adsorbents were determined as a function of adsorbent amount. The results show that newly designed clay adsorbent with polymer addition has great potential (up to 93.5%) to remove Zn(II) ion from waste printing developer.

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THPC1-4

Removal of POPs from industrial wastewater using UVC/H2O2 Photolysis
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In this study UVC-aided TiO2 photocatalytic degradation was applied to remove persistent organic pollutants from industrial wastewater. Experiments were carried out with treated and filtered (0.7 μm) urban wastewater, spiked with AZO Orange II (5mg/l) and 4-Chlorophenol (0.36mg/l). The experimental equipment employed in the assays consists in a tubular reactor of borosilicate glass, (60mm)l operating in a recirculating circuit, irradiated with an UVC lamp (8W). The Hydrogen Peroxide (H2O2) in the process was 200mg/l. The catalyst (TiO2) was immobilized on the external surface of a cylinder of frosted glass (d=40mm).

Experiments have been conducted using a total working volume of 1200ml and a recirculation flow rate of 600 (ml/min).

The efficiency of primary degradation was determined by monitoring the process, for AZO Orange II (absorbance 478 nm) and 4-Chlorophenol compounds. Toxicity assays were also carried out with the aim to evaluate the effects by products. An economic study of this process was included in the global analysis.

THPC1-5

Impacts of advanced wastewater treatment on metal speciation and bioavailability
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In recent years, perfluorinated compounds (PFCs), particularly perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) have been described as compounds of increasing environmental concern and numerous studies were conducted to investigate the environmental distribution and fate of these compounds. This work investigates the transport and fate of PFOA and PFOS in different environmental media (surface water, sewage treatment plants, sediment, and soil) in order to assess the distribution of these chemicals to surface waters or sewage sludge/sewage. The "global average" Kd distribution coefficients based on median concentrations in sediments/surface waters or sewage sludge/sewage. The "global average" Kd value was calculated through the measurement of redox potential (Eh), the reduction of carbonate, nitrate and sulphate, and the removal of dissolved organic carbon. When it was demonstrated that these conditions could be effectively controlled this test system was applied to the removal of test substances under amended conditions.

Conditions were amended in a batch test and in a serum bottle experiment to measure the total removal and mineralisation of two prioritised test substances that have been identified within the biosolids. The results of these experiments were discussed and compared with data obtained from biodegradability experiments performed under unamended conditions following guideline OECD 311.

TU 001

Particle size distribution of airborne perfluorinated compounds
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In recent years, perfluorinated compounds (PFCs), particularly perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) have been described as compounds of increasing environmental concern and numerous studies were conducted to investigate the environmental distribution and fate of these compounds. This work investigates the transport and fate of PFOA and PFOS in different environmental media (surface water, sewage treatment plants, sediment, and soil) in order to assess the distribution of these chemicals to surface waters or sewage sludge/sewage. The "global average" Kd distribution coefficients based on median concentrations in sediments/surface waters or sewage sludge/sewage. The "global average" Kd value was calculated through the measurement of redox potential (Eh), the reduction of carbonate, nitrate and sulphate, and the removal of dissolved organic carbon. When it was demonstrated that these conditions could be effectively controlled this test system was applied to the removal of test substances under amended conditions.

Conditions were amended in a batch test and in a serum bottle experiment to measure the total removal and mineralisation of two prioritised test substances that have been identified within the biosolids. The results of these experiments were discussed and compared with data obtained from biodegradability experiments performed under unamended conditions following guideline OECD 311.

EP06P - Perfluorinated compounds: From emission sources to the place of impact

TU 002

Binding and sequestration of perfluorinated surfactants in soil
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Perfluorinated surfactants (PFSs) produced specifically since the 1950s draw great attention due to their wide application and increasing occurrence in the environment. Sorption coefficients (Kd) and organic carbon normalized soil adsorption coefficients (Koc) are essential for determining soil adsorption behavior and assessing the risk of transfers of these chemicals from contaminated soils to plants and groundwater. We therefore analysed the international literature for concentrations of perfluorinated octanoic acid (PFOA) and perfluorooctanoic acid (PFOS) in different environmental media (surface water, sewage treatment plants, sediment, and soil) in order to assess the distribution and comparison of PFOA and PFOS between these environmental compartments and compared the results with published experimental sorption coefficients. Experimental log Koc values for PFSs for different soils and sediments available in public literature are remarkably similar with a coefficient of variation of only 18%, which should limit the uncertainty of concentrations in drainage water and groundwater calculated from soil concentrations (average log Koc = 2.9). For PFOA however, published sorption coefficients are sparse and the published information regarding the analogy between sorption of carboxylated and fluorinated PFSs is controversial (average log Koc = 2.6).

Our review shows that current pattern of environmental PFOA and PFOS concentrations are strongly influenced by local pollution sources. Nevertheless, we calculated "glider" log Koc values for the different environmental media (groundwater, surface water, sediment, and soil) and compared it with the published experimental sorption coefficients. Experimental log Koc values for PFOA and PFOS concentrations are strongly influenced by local pollution sources. Nevertheless, we calculated "glider" log Koc values for the different environmental media (groundwater, surface water, sediment, and soil) and compared it with the published experimental sorption coefficients.

TU 003
Investigating the sorption of Perfluorinated Compounds in different types of sludge

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Perfluorinated compounds (PFCs) present significant research interest in recent years due to the global distribution, persistence, bioaccumulation and potential toxicity of these compounds. In fact, PFCs are commonly detected in municipal wastewater treatment plants (WWTPs), so far there are few data for their sorption potential to sludge. The aim of this research was to determine the sorption potential of different PFCs to three different types of sludge (primary sludge, secondary sludge and digested sludge). For this reason, sorption experiments were performed with eight PFCs belonging to two different classes; perfluorocarboxylic acids (PFCAs) that contain a carboxyl group in their molecule and perfluoralkyl sulfonates (PFASs) that contain a sulfonate group in their molecules.

At first, sorption experiments were performed to investigate the equilibrium time for each target compound and the role of sludge inactivation on sorption potential of PFCs. For this reason, samples were taken at different time intervals and PFCs were detected in dissolved and particulate phase. PFCs were extracted by solid-phase extraction (SPE) and then analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using an on-line SPE-HPLC-MS-MS system.

Two seasonal sampling campaigns were performed and samples were taken in seven consecutive days in each sampling campaign. The analytes were extracted by solid-phase extraction (SPE) and then analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using an on-line SPE-HPLC-MS-MS system.

Analyses of PFAS were achieved by using an on-line SPE-HPLC-MS-MS system. Recovery for PFASs for all analytes was above 70% at µg/L levels. Limits of detection (LOD) ranged from 0.2 to 2.5 ng/l.

All in all the above mentioned substances degraded to PFOA or PFOS in ranges from 1 to 8 per cent during 14 days. The lowest metabolic rate was monitored for PFOSA. In addition the technical product degraded to perfluorohexanoic acid (PFHxA) as well. The absence of degradation products in the abiotic controls show that the metabolism has to be attributed to microbial degradation only.

Acknowledgements

"This project was implemented under the Operational Program «Education and Lifelong Learning» and funded by the European Union (European Social Fund) and National Resources - HRAKLEITOS II."
Occurrence of 14 perfluorinated compounds and other priority and emerging organic compounds in fishes from the Rhone River (France)  

The main goal of this study was to obtain data on the occurrence and levels of 34 priority and emerging organic compounds in freshwater fish sampled in the Rhone River. The analyses included 14 perfluorinated compounds (PFCs), and also 5 alkylphenols (AP), bisphenol A (BPA), 3 hexabromocyclododecanes (HBCCD), 9 polybromodiphenylethers (PBDE) and 2 compounds, hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD) for which Environmental Quality Standard (EQS) have been set for freshwater by the Water Framework Directive (2000/60/EC) and other European Directives. Since the ubiquitous occurrence and potential toxicity to human beings, the perfluorinated compounds (PFCs) have attracted more concern in the recent years. In 2010, one of the PFCs, perfluorooctanoic acid (PFOA) was added to Annex B of Stockholm Convention on Persistent Organic Pollutants (POPs). Recently, more subcategories, i.e. perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFHxA), were widely used and the elevated levels have been found all over the world. In this study, four cruises in Elbe River and three cruises in North Sea were conducted throughout the whole year of 2011 to investigate the seasonal distribution of perfluorinated compounds in perfluorinated compounds in surface water. Perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonates (PFSAs) were determined in both particle and dissolved phases in more than 100 samples. The profile patterns suggested the industrial and urban discharge which were the major source of PFCs in river and coast water.

TU 001  
Distribution of perfluorinated compounds (PFCs) in surface water from Elbe River and North Sea, Germany  

Knowledge about sources of PFA to drinking water is currently scarce and focuses on surface water based drinking water. Waste water treatment plants have been identified as major sources for PFA to surface waters and ultimately to the drinking water produced from it. However, groundwater is in many countries the major source for drinking water production (in NL 6%). The extent of contamination of groundwater by PFA and the contamination pathways to groundwater bodies remain largely uncertain. In the work presented, we identified different direct sources of PFA to groundwater within a catchment area of a public supply well field (PSWF) in the Netherlands. It was found that landfill leachate, and urban/military base affected rainwater contaminated the groundwater within the catchment area of the PSWF. Rainwater not affected by the direct sources also infiltrated in this area and provided a background contamination to the groundwater.

Maximum concentrations encountered in the landfill leachate plume were 17.5 ng/L perfluorooctanoic acid (PFOA) and 11.6 ng/L perfluorobutanolic acid (PFBA). Maximum concentrations measured in the groundwater halfway the contaminated sources and the PSWF (15 years travel distance) were 29 and 161 ng/L for PFOA and PFBA respectively. Concentrations in the groundwater wells at a travel distance of 25 years were much lower: 0.96 and 3.5 ng/L for PFOA and PFBA respectively. The chemical signature of the groundwater showed that infiltrated rainwater, the landfill leachate and the infiltrated urban/military water contaminated the abstracted water. Based on the hydrological modeling it was shown that 1% of the treated groundwater was originating from the landfill and military area and 99% from background contamination. This is seen in the relative abundance profile of the PFA in the pumped groundwater.

TU 009  
Seasonal distribution of perfluorinated compounds (PFCs) in surface water from Elbe River and North Sea, Germany  

TU 010  
Occurrence of 14 perfluorinated compounds and other priority and emerging organic compounds in fishes from the Rhone River (France)  

The monitoring campaigns allowed to identify hot spots in the main Italian basins.

Unlike rivers Severe and Adige which are not significantly impacted, concentrations determined at the basin closure of the river Po are comparable to those measured in the PFAS heavily impacted areas of northern Europe: PFOA concentrations are low (ranging from < LOD to 2.5 ng/L) while PFOS is the main compound (about 25 ng/L). Elsewhere, the impact of different factories in the Tanaro/Bormida watershed, was confirmed as the main source of the latter molecule. Plant for the production of fluorochemicals, used as intermediates in the polymer synthesis, is a significant source of PFOA and PFBS in the river Brenta which discharges in the Adriatic Sea. Two important textile industrial districts in Italy (counties of Vicenza and Prato) have been identified as also a significant source of PFPeA and PFHxSA which are discharged in the River Po and in river Arno. These sources of PFA are considered as tracers for this kind of industrial pressure. The same compounds were also measured in the river Adda, an important tributary of the river Po, but the source shall be still to be identified.

The diffusion of these substances in the drinking waters in the same areas has also been studied.

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Therefore, the feasibility of feathers for PFCs monitoring should be investigated more in depth in the future.

Overall, these results indicate that PFOS levels in feathers and liver are highly correlated, while PFOA levels in feathers are probably originating from external contamination.

Perfluorinated chemicals (PFCs) in feathers and tissues of barn owls (Tyto alba), collected in the province of Antwerp (Belgium). A major PFC plant (3M) is located in the close vicinity of the city of Antwerp and levels of PFCs in biota from this area have been found to be very high in previous studies. We analysed levels of PFCs in tail feathers and tissues (liver, muscle, preen gland, adipose tissue) obtained from barn owl road-kill victims (n=15). We used methods for left-censored data to cope with levels below the limit of detection. We aimed to study the main sources for PFCs in feathers as levels can both originate from internal sources (via the blood) as from external contamination (via e.g. air, dust). PFOS was detected in all tissues (median concentration: 135 ng/g in muscle, 305 ng/g in liver, 431 ng/g in preen oil, 203 ng/g in adipose tissue), also in feathers (median 14.7 ng/g (2.2-56.6). PFOS levels in feathers and liver tissue were highly correlated (r=0.79, p<0.001), but this was not the case for PFOA levels between feathers and muscle (r=0.15, p=0.47) or between muscle and liver (r=0.46, p=0.08). This may be due to the chemical properties of PFCs which are mainly bound to proteins in the blood, thus reducing the suitability of muscle tissue. MCAO was measured at high levels in the feathers (<14.1-670 ng/g), but not in the tissues (more than 50% LOD). Furthermore, FFOA levels in feathers and liver were not significantly correlated (r=0.17, p=0.37). These results suggest that PFOA may be present on the external surface of the feathers and that was not washed off by using distilled water and hexane. External contamination with PFOA is most probably originating from the air due to the close vicinity of potential point sources. PFHxS could only be quantified in liver and preen oil. Sporadically other PFC compounds were detected as well, mostly in liver. Overall, these results indicate that PFOS levels in feathers and liver are highly correlated, while PFOA levels in feathers are probably originating from external contamination. Therefore, the feasibility of feathers for PFCs monitoring should be investigated more in depth in the future.

Monitoring of perfluorinated compounds
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Because of their persistency and widespread range of application perfluorinated compounds (PFCs) are found as ubiquitous contaminants in aquatic and terrestrial organisms as well as in food products. HPLC-MS/MS analysis of animal and non-animal foodstuffs and wild game was performed within the framework of preventative consumer protection.

Fish from regional waters:
Sixteen fish from the Rhine river including eel, perch, roach, tench and catfish were examined for the presence of PFC. Concentrations between 5.2 µg/kg and 143 µg/kg were detected in muscle tissue as the sum of PFC. (PFBS, PFPeA, PFHxS, PFHpA, PFOA, PFOS, PFNA, PFDA, PFDoA). The highest concentration was measured in perch muscle tissue.

Roe deer:
Use of PFOA has been declining since the year 2000. In order to observe the ensuing temporal changes in PFC concentrations in animal matrices a retrospective study of liver tissue from roe deer was undertaken. A total of 110 samples taken between 1998 and 2010 from the German Environmental Specimen Bank were examined. A decline in PFOS contamination since the beginning of this millennium is evident. Average PFOS concentration was 9.2 µg/kg in the year 2000 and 1.9 µg/kg in 2010.

Wild boar:
PFOS and PFOA concentrations were measured in 506 muscle tissue samples and 529 liver samples from wild boar. The arithmetic mean of PFOA concentrations detected in muscle tissue was 1.38 µg/kg whereas the mean PFOA concentration was below the LOQ. In liver tissue the mean PFOA concentration was 4.02 µg/kg (Maximum value 45 µg/kg) (1).

Foodstuffs:
Measurements of 82 samples of French fries showed PFC concentrations above the LOQ in 3 samples. No concentrations above the LOQ were detected in 30 samples of ice cream, 14 samples of whole milk, 19 samples of carrots or 16 samples of grains. A total of 84 samples of ocean fish, farmed fish, seafood, fish in packaged salads and canned tuna were tested for the presence of PFC. No PFC were detected in 82 of the samples. Two carp from fish farms had 2 and 14 µg/kg, respectively in muscle tissue.

(1) T. Stahl, F. Falk, K. Failing, J. Berger, S. Georgii, H. Brunn, Article title: PFOS and PFOS in Liver and Muscle Tissue from Wild Boar in Hesse, Germany, Arch Environ Contam Toxicol, DOI: 10.1007/s00244-011-9726-3

TU 015 Development of a pharmacokinetic model (PBPK) for the assessment of infant exposure to PFOA and PFOS for health risk assessment
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Perfluorinated compounds (PFCs) are very stable compounds with a lot of industrial application, but also very persistent on the biota and the environment. Among them, perfluorooctane sulfonate and PFOA (perfluorooctanoic acid), have a long persistence in the human body (4-6 years). This persistence creates an important concern among the scientific community and the health authorities. In fact, PFOS was listed under the Stockholm Convention in May 2009 as a persistent organic pollutant (POP), while PFOA is a serious candidate to enter that list. The main target organs of accumulation are blood and breast milk. Thus, PFOA can be a risk for adult population and infant. In order to assess the tissue concentration, PBPK models are able to assess the concentration along the time in the main target organs of concern. PBPK models are mathematical representations of the human body where the organs are considered as compartments, and they can be resolved computationally as a set of equations. The PBPK model presented here is a multi-compartmental model for PFOA and PFOA for adult and childhood exposure during the breastfeeding period. Aside from the blood and breast milk, another tissue such as brain and kidney have been considered. PFCs are well absorbed, non metabolized and eliminated by urine and breast milk. The main sources of exposure are adult population and food and water intake. The data for food and water ingestion have been obtained from a large monitoring study in Catalonia, Spain. For toxicity, the tissue residue approach (TRA) was used. The TRA is the use of tissue concentrations as the dose metric for characterizing toxicant potency. The PBPK model has been validated by comparing theoretical values with experimental data of PFCs chemicals in blood serum and breast milk collected in Catalonia, Spain. Finally, uncertainty and sensitivity analyses have been performed. A normalized local sensitivity analysis was performed on the model to examine the influence of each model parameter on the model output. Sensitivity coefficients were calculated for the predicted plasma area under the curve (AUC; total concentration) with the original parameters and for those resulting from a low change in each parameter value.

TU 016 Acute toxicity of perfluorinated compounds to two kinds of cladocerans
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Due to their unique properties, poly- and perfluorinated compounds (PFCs) have been manufactured and used during the past 50 years in a variety of industrial and commercial products, such as household surface finishes, food packaging, water- and stain-resistant materials, and fire-fighting foams. PFCs were released to the environment during production, usage and disposition. PFCs tend to persist in surface waters. Therefore their toxicity to aquatic organisms is of particular concern. Serving as a food source for fish, mammals and other aquatic organisms, cladocerans are one of the key trophic elements of aquatic ecosystems. In addition to their ecological significance, cladocerans have the advantages of being useful as test organisms due to their short life cycle, their ease of laboratory culturing, their limited space and water volume requirements, and their sensitivity to chemicals. Cladoceran species are therefore widely used in aquatic toxicology. Among freshwater cladocerans, Daphnia magna is probably the most commonly used test organism in ecotoxicological studies. Some toxicity tests have been performed on cladocerans for perfluorosulfonate sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) or their salts.
The results indicated that, low concentrations of these chemicals generally caused malformations in the skeletal system. It was observed that high concentrations (10 mg/L) caused 100% mortality. In the Fish Embryo toxicity test, a Sexual Development Test (SDFD) was conducted and effects on vitellogenin (VTG), body and liver somatic indices and gonad histopathology including sex ratios were evaluated. A reproduction test (Fish Screening Assay: FSA) was also performed where effects on reproductive parameters like spawning, fecundity and fertilization rate were evaluated, as well as effects on histopathology, liver enzyme activity VTG-levels and key gene expression levels in the adult fish.

The relation between LogPow of perfluorinated carboxylates (PFCAs=C4, C5, C6, C7, C8) and immobility ratio of Daphnia magna was examined. As the carbon number increased across all remaining treatments ranged between 95 and 100%. In terms of concentrations, the NOEC and LOEC for post-hatch larval survival until Day 28 were both 10 mg/L respectively. For fish total lengths, the NOEC and LOEC determined on Day 28 post-hatch were 10 mg/L respectively. For fish dry weights, the NOEC and LOEC determined on Day 28 post-hatch were both >10 mg/L respectively. In conclusion, PFCs with short chain lengths should be primarily considered throughout the development of new perfluorinated alternatives.

Only PFOA caused significant effects on hatching rate and success. The effects of PFOS, PFOA and PFBS on the heart rate at 48 hpf could be the result of effects on intrinsic processes that control the heart rate, while altered heart rates at 72 hpf can be attributed to the shorter body lengths. In conclusion, PFOS with short chain lengths should be primarily evaluated with the partition coefficients. According, the correlation between the partition coefficients and effects on Daphnia magna. Also, we studied the effects of C6, which is available for industrial use, on development of fertilized eggs of rainbow trout.

The relation between LogPow of perfluorinated carboxylates (PFCas=C4, C5, C6, C7, C8) and immobility ratio of Daphnia magna was examined. As the carbon number increased for C4 and C5, immobility rate became 100% in 24 hours, while C6 reached 100% in 48 hours. The immobility rates of Daphnia magna for C6 and C7 were at the same level. For PFCCs, no correlation between the carbon number and immobility was observed. In fish early life stage toxicity test of C6, hatching success in the control group was 74%, satisfying the validation criterion for hatching success (>66%). The NOEC and LOEC determined on Day 28 post-hatch survival until Day 28 were both considered to be equal to or greater than 10 mg/L.

For fish total lengths, the NOEC and LOEC determined on Day 28 post-hatch were 10 mg/L. For fish dry weights, the NOEC and LOEC determined on Day 28 post-hatch were >10 mg/L. All validity criteria were satisfied during the test, therefore the test was considered to be valid. C6 proved to have no adverse effects on hatching rate and success. In the present study, the adverse effects of these chemicals on these two cladocerans decreased with increasing fluorinated carbon chain length (nC) and quantitative structure-activity relationships were developed to predict the toxicity of the chemicals. This resulted in the following order of toxicity: PFOA>PFOA>PFOA>PFOA. Furthermore, exposure to the different PFCs resulted in some general effects, such as deformations of the tail and an uninfated swim bladder, as well as in more specific effects depending on the structure of the tested chemicals. Oedemas and effects on length could only be detected in 8-carbon PFCs while malformations of the head were a more specific action of the sulfonated PFCs.

Our environment has been affected by increasing concentration of organic and inorganic pollutants from anthropogenic sources. Perfluorinated compounds are important source of pollution and they are major risks for the aquatic ecosystems. Perfluorooctane sulfonate (PFOS) and Perfluorooctane sulfonyl fluoride (POSF) are both industrially synthesised perfluorinated chemicals and they produced in substantial amounts. They are found widely in surface water and aquatic sediments. In this study, the comparative embryotoxic effects of perfluorobutane sulfonate (PFBS) and perfluorobutanoic acid (PFBA) were examined in zebrafish embryos (Danio rerio) during 120 hours post fertilization (hpf). The different chain lengths and functional groups of the selected chemicals made it possible to determine the structure-activity relationship of these compounds. PFCs with longer chain lengths (C8) tend to be more toxic than PFCs with shorter chain lengths (C4). Comparison based on the functional groups of compounds with the same chain length indicates that PFCs with a sulfonate group have a larger toxic potential than the ones with a carbonyl group. This resulted in the following order of toxicity: PFOA>PFOA>PFOA>PFOA. Exposure to the different PFCs resulted in some general effects, such as deformations of the tail and an uninfated swim bladder, as well as in more specific effects depending on the structure of the tested chemicals. Oedemas and effects on length could only be detected in 8-carbon PFCs while malformations of the head were a more specific action of the sulfonated PFCs. Only PFOA caused significant effects on hatching rate and success. The effects of PFOS, PFOA and PFBS on the heart rate at 48 hpf could be the result of effects on intrinsic processes that control the heart rate, while altered heart rates at 72 hpf can be attributed to the shorter body lengths. In conclusion, PFOS with short chain lengths should be primarily evaluated with the partition coefficients. According, the correlation between the partition coefficients and effects on Daphnia magna. Also, we studied the effects of C6, which is available for industrial use, on development of fertilized eggs of rainbow trout.

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For fish total lengths, the NOEC and LOEC determined on Day 28 post-hatch were 10 mg/L. For fish dry weights, the NOEC and LOEC determined on Day 28 post-hatch were >10 mg/L. All validity criteria were satisfied during the test, therefore the test was considered to be valid. C6 proved to have no adverse effects on hatching rate and success. In the present study, the adverse effects of these chemicals on these two cladocerans decreased with increasing fluorinated carbon chain length (nC) and quantitative structure-activity relationships were developed to predict the toxicity of the chemicals. This resulted in the following order of toxicity: PFOA>PFOA>PFOA>PFOA. Furthermore, exposure to the different PFCs resulted in some general effects, such as deformations of the tail and an uninfated swim bladder, as well as in more specific effects depending on the structure of the tested chemicals. Oedemas and effects on length could only be detected in 8-carbon PFCs while malformations of the head were a more specific action of the sulfonated PFCs. Only PFOA caused significant effects on hatching rate and success. The effects of PFOS, PFOA and PFBS on the heart rate at 48 hpf could be the result of effects on intrinsic processes that control the heart rate, while altered heart rates at 72 hpf can be attributed to the shorter body lengths. In conclusion, PFOS with short chain lengths should be primarily evaluated with the partition coefficients. According, the correlation between the partition coefficients and effects on Daphnia magna. Also, we studied the effects of C6, which is available for industrial use, on development of fertilized eggs of rainbow trout.

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EP07P - Plastics: an emerging risk to the marine environment

TU 022

Plastic debris and toxin releases in the Pacific Ocean
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Plastics comprise the majority of collected waste in worldwide beach cleanups in 2006, 2007, and 2008. In California, Washington, Oregon, and Hawaii the five most common plastic debris items on beaches are cigarette filters, food wrappers and containers, beverage caps and lids, bags, and food service items, e.g., cups, plates, and cutlery. The majority of plastic items are made from four common plastics; polyethylene, polypropylene, polystyrene, and PET, accounting for 75% of the plastic debris. Pre-consumer plastics are generally not found for the same amounts of plastics in the oceans can lead to fragmentation and result in small plastic particles that can degrade and release toxic chemicals such as phthalates, flame retardants, BPA, antimony oxide, heavy metal nitrates, and styrene monomer as the plastics break down. Plastics can accumulate toxins floating in the oceans from persistent organic pollutants (POPs). POPs can include DDT, hexachlorobenzene, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons, among others.

TU 023

Micro-plastics in the marine environment - a global assessment
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Institute of Environmental Studies (IVM), VU University, Amsterdam, Netherlands

GESAMP has set up a new Working Group entitled 'Sources, fate and effects of micro-plastics in the environment - a global perspective'. With multi-agency support and with a multi-disciplinary membership, it is reviewing a wide range of information about the supply of plastics and microplastics to the ocean, and the biological, chemical and physical processes with which plastic debris and microplastics interact. Ingestion by marine organisms is an important process in the food chain and an essential aspect of plastic debris recycling in the oceans. The Working Group is gathering evidence on the extent of ingestion by sea animals and is producing a global report on the effects of ingestion in marine environments. The Working Group has identified the need to monitor the distribution, abundance and effects of microplastic debris in the marine environment. While data is accumulating rapidly, there is a need for standardization of methods and the establishment of global reference points, so that trends can be monitored over time and space. A strategy for achieving this is being developed in cooperation with the food science community. The Working Group is also considering the need for collecting standard sets of samples from different regions and for the development of methods for analysing samples for microplastics.

TU 024

Marine micro litter under the marine strategy framework directive - science and policy
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University of Plymouth, Plymouth, United Kingdom

Plymouth University School of Marine Science and Engineering, Plymouth, United Kingdom


"Trends in the amount of litter washed ashore and/or deposited on coastlines, including analysis of composition, spatial distribution and, where possible, source (10.1.2)

"Trend in amount and composition of litter in the water column (including floating at the surface) and deposited on the sea-floor, including analysis of its composition, spatial distribution and, where possible, source (10.1.2.1)

"Trends in the amount, distribution and, where possible, composition, micro-plastics (10.1.3)

"Trends in the amount and composition of litter ingested by marine animals (e.g. stomach analysis) (10.2.1).

A technical working group has prepared technical recommendations in support of the implementation of the directive by EU Member States. The group identified options and available tools for the monitoring of Marine Litter and developed a roadmap showing the necessary next steps in preparing the implementation of the directive. The poster is presenting specific information regarding marine micro litter under the MSFD.

TU 025

Using the continuous plankton recorder to determine the abundance of microplastic debris in the subsurface marine waters
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Sir Alistair Hardy Foundation for Ocean Science (SAHFOS), Plymouth, United Kingdom

Fragmentation of plastics debris into "microplastic" pieces is an emerging issue of concern. Our knowledge of distribution and accumulation of microplastics is patchy and in order to quantity and monitor the distribution and in particular temporal trends there is a need for a broad sampling programme and standardised methods. The aim of this research is to study the spatial-temporal abundance of microplastic debris in ocean surface waters and our preliminary investigation of microplastic contamination in Continuous Plankton Recorder (CPR) samples reported by analysts at the Sir Alistair Hardy Foundation for Ocean Science (SAHFOS) has confirmed the presence of synthetic plastics in several European locations (North Sea, Irish Sea, English Channel and the North Atlantic). We used Fourier Transform Infrared (FT-IR) spectrometry to identify the known pieces. The most common plastic types were Polyethylene terephthalate (PET) followed by Nylon and Acrylic but mean abundance was typically less than one item per cubic meter of sea water.

TU 026

Microplastics in personal care products from Brazil is this source relevant to the aquatic contamination?
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Plastics have been recently incoporated into the group of emerging pollutants due to their wide distribution in marine and coastal environments all over the world, as well as their persitency and hazard to the wildlife. Moreover, they have highly diverse diffuse sources and high capacity of distribution. In addition to other sources, microplastic (< 5 mm) can enter marine environment in frequencies of skin cleaners and others personal care products reported in a result, this specific class of plastic has been found containing sediments and pelagic zones. Despite the effects related to direct ingestion, microplastic might also transfer adsorbed organic contaminants (OCs) to aquatic organisms. In the current study, particles from 18 of the most frequently used personal care products were characterized regarding amount, size, form and composition. Analysis was performed by suspension and filtration of plastic particles, followed by microscopic analysis and weight of the particles. Undergoing chemical analysis by Fourier transform infrared spectroscopy (FTIR) will confirm the apparent predominance of polystyrene among plastics. The amount of plastic ranged from 1.3 to 103.1 mg/m2 of product, whereas the distribution frequency ranged from 100 to 400 microns. Due to methodological limitations, only 10 products had their size measured. Most of the plastics have irregular shapes, although spherical particles were also seen in some products. The preliminary results pointed out this as a relevant source of microplastics to the environment. However, this physic-chemical characterization of personal care products is essential to assess their potential to adsorb, concentrate and transfer OCs, improving somehow their bioavailability from the environment.

TU 027

International Pellet Watch : background levels, hot spots, legend and distribution trends
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Surfrider Foundation, Oahu, United States of America

International Pellet Watch (IPW) is a volunteer-based global monitoring program designed to monitor the pollution status of the oceans and to understand the risks associated with plastics in marine waters. Based on the Bakken sediment plume off New York Bay, background levels of persistent organic pollutants (POPs) have been tentatively determined. By comparison of POPs concentrations in pellets from industrially-developing countries (e.g., Ghana) with the background levels, PCB inputs from wastes were suggested. Through global comparison, hot spots of PCB pollution were identified, e.g., Le Havre (France), Athens (Greece), Tokyo Bay (Japan), Sydney Harbor (Australia), Los Angeles, San Francisco, Boston Harbor, New York Bay to Lake Erie (USA), Santos Bay (Brazil). Combination of IPW with passive air sampling indicates that legacy pollution is major contributor to most of the hot spots of PCB pollution. In addition to the spatial variation, pellet watch was applied to reveal temporal trend in POPs pollution in California coast and Tokyo Bay.

TU 028

Partitioning between water and plastics of polychlorinated biphenyls in marine animals
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The presence of plastic debris in the oceans is a potential hazard for marine animal species in several ways. It is not only the plastics themselves that form a potential hazard. Chemical additives may be added during plastics production in order to catalyse monomers into polymers and give it different properties. Some of these additives have been associated with carcinogenic and endocrine disrupting effects. Moreover, due to its hydrophobic nature, plastic debris can be a hazard to marine animal species because of accumulation of organic contaminants. An important hazard are the persistent organic pollutants (POPs), which do not completely dissolve in water and do not degrade into other compounds. With a short timeframe, it will provide an evidence base for use by policy makers, regional bodies a other stakeholders, and help to develop research priorities. The poster will describe the Terms of Reference, work programme, membership and intended outcomes of the group, and will issue an invitation to SETAC Europe participants to become involved in this initiative.
Our study focused on the occurrence of plastic debris in marine animals. These organisms are at the bottom of the food chain, and through the ingestion and uptake of microplastics, they are exposed to a wide range of pollutants. This exposure can have significant effects on their health and survival, as well as on the marine ecosystem as a whole.

In this study, we examined the presence of microplastics in the digestive tract of several species of animals, including sea turtles and mussels. We found that a large number of plastic was present in the intestines of more than 60% of the animals we studied. The main categories of plastics found in the specimens were fragments, followed by debris on the sea floor and foamed and fragment user plastics. Debris physical characteristics such as weight, length, hardness, and colour were also noted.

The first result obtained from this study was that a large number of plastic was found, prevalently into the intestine, in more than 60% of the animals. The main categories of plastics found in the specimens were fragments; moreover, in some specimens, we found little amounts of floating debris more than by debris on the sea floor. The second type of plastics found in the specimens were fragments; moreover, in some specimens, we found little amounts of floating debris more than by debris on the sea floor.

Although the 10 µm-particles were detected in much smaller quantities than expected based on the observations of the other particle sizes, i.e. 8 times less than the 30 µm-particles, there was a significant increase in their concentration in the faeces, with a value of 110 particles/mL. Analysis of the faeces demonstrated that M. edulis filtered and ingested all particle sizes in the same ratio as they were added to the seawater (i.e. 5/1). However, only the 30 and 90 µm-particles were detected in the faeces, and the 30 and 90 µm-particles were detected in the same ratio as they were added to the seawater (i.e. 5/1).

The uptake of microplastics in the mussel was size-selective and has an adverse effect on the energy allocation in the mussel. The energy consumed to filter and feed on particles was significantly higher for the 10 µm-particles than for the 30 and 90 µm-particles. This resulted in a decrease in the growth rate of the mussels, and a decrease in the energy allocation to reproduction.

The ingestion and uptake of microplastics showed no significant effects on cellular energy allocation. Exposure to plastic did not significantly increase the metabolic rate of the mussels. However, the ingestion and uptake of microplastics did affect the growth rate of the mussels, with a significant decrease in the growth rate observed for the 10 µm-particles.

The presence of high concentrations of microplastics, which was approximately 100 times the environmentally relevant concentration, could have significant short-term adverse effects on the infection and translocation of other pollutants. Long-term exposure studies could help us to further understand the effects of ingestion and uptake of microplastics in Mytilus edulis.
The southern hemisphere’s elevated UV light exposure encourages the year-round use of UV protective chemicals (UV filters and UV light stabilizers) in personal care and plastic products. UV filters and preservatives are known to be hormonally active in vitro and/or in vivo, raising concerns of possible endocrine disrupting effects in the environment. There is little published data of occurrence of those compounds in Australian aquatic environments. This study is the first investigation of UV protective chemicals in environmental waters in Victoria, specifically in one of Melbourne’s estuaries. Water samples were taken from four sites (A - D). Screened compounds included 11 UV filters, 12 UV stabilizers, 12 preservatives and the metabolite, and one fragrance, including commonly used compounds in Australia such as 4-MBC, EHCMS, octocrylene, UV-328, HEB, 2-phenoxethanol, methyl paraben, and propyl paraben. Water samples were extracted for polar and relatively non-polar compounds using two different types of solid phase extraction methods: Oasis HLB (Waters Corp.) for polar compounds, Empore C18FF (3M) for relatively non-polar compounds, then analysed by GC-MS, after isolation of the screened compounds. Samples matched the screened compound profiles. Site D had a very different contaminant profile, with very high concentrations of the screened compounds. Sites A, B, C, samples had similar compound profiles. The question arises how different activated sludges are regarding these parameters, how large the adsorption to sludges varies. The sorption of chemicals to sorbents like soils or sediments typically depends on several parameters of the sorbent (e.g. pH, ion-exchange capacity, redox potential, organic content, clay content, texture) . The question arises how different activated sludges are regarding these parameters, how large the adsorption to sludges varies. The effects observed in the ecotoxicity tests with the macro alga (EC50s in the range of 7.5 to 46%) and the crustacean (LOECs in the range of 11.25 to 90%) could not be assigned to either pharmaceutical residues or metals, but in general showed that these treatment facilities release water with a relatively low toxic potential, comparable to water that have been treated with advanced treatment technology. From the present study it can be concluded that constructed wetlands may provide a complementary sewage water treatment option, especially where other treatment is lacking today. To fully remove micro-pollutants from sewage effluent however, other more advanced treatment technologies are likely needed.

TU 035

A comprehensive study of activated sludges and sources and fate in a highly urbanized and inhabited area in Italy

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Pharmaceuticals are considered emerging contaminants of particular concern, as many of them display biologically active properties (e.g. hormone disruption) and have been found in the environment, including in sludge. The potential risk of human pharmaceuticals to the environment has to be determined, e.g. according to the EMEA Guideline EMEA/CHMP/SWP/4447/00. This guideline focuses on the use of pharmaceuticals and the entry into the environment via excretion and wastewater effluent to a sewage treatment plant (STP). It is assumed that a substance with high sorption to activated sludge may reach terrestrial ecosystems by land spreading of sewage sludges. The adsorption to sludges has therefore to be tested as an additional guideline in an adsorption study. A terrestrial risk assessment must be completed with the drug if its adsorption coefficient to sludge exceeds a defined trigger value (Koc of 10000 L/kg or Kd of 3700 L/kg) and the drug is not readily biodegradable. The sorption of chemicals to sorbents like soils or sediments typically depends on several parameters of the sorbent (e.g. pH, ion-exchange capacity, redox potential, organic content, texture). The question arises how different activated sludges are regarding these parameters, how large the adsorption to sludges varies. Thus the investigation was conducted in waste, surface and ground water. Composite samples were collected, filtered, enriched with internal standards and solid-phase extracted. LC-ESI/MS/MS with multiple-reaction monitoring and stable-isotope dilution assay was used for quantitation. Recoveries were generally higher than 80% and limits of quantification were in the low ng/L range. Ciprofloxacin, ofloxacin, atenolol, furosemide, hydrochlorothiazide, clarythromycin, ibuprofen, ketoprofene, diclofenac, naproxen, and carbamazepine were the most abundant residual drugs in urban wastewater. Their removal in conventional WWTPs was generally lower than 50%, with no removal for bezafibrate, hydrochlorothiazide, furosemide and carbamazepine. Removal rates were also affected by the type of advanced treatments adopted (i.e. disinfection, UV treatment) in the plants investigated. The amounts of pharmaceutical active substances discharged in the environment daily through treated wastewater ranged between 1.2 and 2 kg. The total amounts of the investigated pharmaceuticals in the rivers flowing through the area investigated ranged between 0.9 and 2.7 kg/day respectively before and after the city of Milan. Traces of pharmaceutics were found in the low ng/L range also in untreated ground water. Moreover, the pattern of contamination of ground water was higher in sites closer to rivers, suggesting the contribution of surface water to the contamination. This investigation allows the quantification of the mass flow of these substances flowing through a highly urbanized and industrialized area.

TU 036

An analysis of free water surface wetlands as tertiary sewage water treatment of micro-pollutants

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Umé University, Umeå, Sweden

Increased attention is currently directed towards potential negative effects of pharmaceuticals and other micro-pollutants discharged into the aquatic environment via municipal sewage treatment plants (STPs). Effluent micro-pollutant concentrations and removal efficiencies have therefore been suggested as promising tools for improving the removal efficiency of pharmaceuticals in existing Sewage Treatment Plants (STPs). Constructed wetlands are also capable of removing a variety of micro-pollutants, including some pharmaceuticals, and could hence be a resource efficient complement to more advanced treatment technologies. The purpose of the present study was therefore to increase the knowledge about the potential use of constructed wetlands as a treatment step to reduce emissions of organic micro-pollutants from municipal sewage effluents. Under cold winter conditions, incoming and outgoing waters from four Swedish free water surface wetlands, operated as final treatment steps of sewage effluent from municipal STPs, were sampled and analyzed for levels of a set of 92 pharmaceuticals and 22 inorganic components as well as assessed using subchronic ecotoxicity tests with a marine alga as a test organism.

Sixty-five pharmaceuticals were detected in the range 1 ng/L to 7.6 µg/L in incoming and outgoing waters from the four investigated wetlands. Although the sampling design used in the present study lacks the robustness of volume proportional 24 h composite samples, the average estimated removal rates ranged from 42 to 52%, which correspond to removal efficiencies (E50%) in the range of 7.5% to 46%. The presented results are comparable to previous published values. The effects observed in the ecotoxicity tests with the macro alga and a crustacean .

TU 037

Large screening of pharmaceuticals and hormones in sludge based on LC-ToF-MS

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Ecosystems are potentially contaminated by many chemical substances from domestic activities, through the spreading of treated sewage sludge. However, environmental and health risks associated with their use. This is partly explained by the difficulties of detection and quantification of such pollutants in the solid phases. It is, indeed, a real analytic challenge given the diversity of analytes, their presence generally in trace amounts and the complexity of the matrix which makes difficult the extraction step. Thus it appears that the development of environmental monitoring and the complexity of the matrix which makes difficult the extraction step. Thus it appears that the development of environmental monitoring and the complexity of the matrix which makes difficult the extraction step. Thus it appears that the development of environmental monitoring and the complexity of the matrix which makes difficult the extraction step.

In this context, the aim of the study was to develop a method for the large screening of pharmaceuticals and hormonal steroids in sludge. Therefore, nearly two hundred compounds were analyzed by liquid chromatography and mass spectrometry. The analysis of complex matrices such as sewage sludge needed a rigorous sample preparation to obtain a repeatable and enough sensitive analysis to achieve the detection limits required. For this purpose, an extraction step using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was set up. The method was successfully applied to various kinds of sludge (limed, digested, dried, liquid, composted) collected in several sewage works in France.

TU 038

Adoption of human pharmaceuticals to activated sludge: how many different sludges should be tested for an environmental risk assessment?

U. Memmert

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The European Medicines Agency (EMA) has provided a guideline for the determination of the pharmaceuticals in sewage sludge that have to be determined, e.g. according to the EMEA Guideline EMEA/CHMP/394/447/00. This guideline focuses on the by the use of the pharmaceutical and the entry into the environment via excretion and wastewater effluent to a sewage treatment plant (STP). It is assumed that a substance with high sorption to activated sludge may reach terrestrial ecosystems by land spreading of sewage sludges. The adsorption to sludge has therefore to be tested as an additional guideline in a sorption study. A terrestrial risk assessment must be completed with the drug if its adsorption coefficient to sludge exceeds a defined trigger value (Koc of 10’000 L/kg or Kd of 3’700 L/kg) and the drug is not readily biodegradable. The sorption of chemicals to sorbents like soils or sediments typically depends on several parameters of the sorbent (e.g. pH, ion-exchange capacity, redox potential, organic content, texture). The question arises how different activated sludges are regarding these parameters, how large the adsorption to sludges varies, and consequently how many sludges should be tested for a safe risk assessment. The number of sludges to be tested is not clearly defined in the guideline EMEA/CHMP/SWP/4447/00. In the present study, the results of batch equilibrium sorption tests with different sludges will be shown for several pharmaceuticals. The variability of the adsorption coefficients, obtained for different sludges by the same analytical method and within the same laboratory, will be discussed in the context of the trigger values used in the environmental risk assessment.

TU 039

Analysis of antimalarial drugs in water

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Malaria is a general illness in many countries and therefore antimalarial drugs are prescribed in great quantities. There are more than 20 different molecules being used today. To fully remove micro-pollutants from sewage effluent however, other more advanced treatment technologies are likely needed.

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for treatments. These drugs are excreted mainly via urine. As a result, they may reach the environment after waste water treatment, trough yellow water used as a fertilizer or by direct discharge. There is limited information on the fate of antimalarials in the environment. It is believed that some of these drugs are rather persistent due to their organohalogen moieties. Hence, the fate of these drugs in the environment might be a subject of concern.

To date, many methods to analyze antimalarial drugs in blood and urine have been published. However, only two methods have been published to analyze a few antimalarial drugs (artemisinin derivates and chloroquine) in water or soil. For that reason, the first multi-residue method for the analysis of antimalarial drugs and their metabolites in water has been developed. The analytical process combines SPE with LC-MS/MS and can be further developed to analyze soil samples. The presented method can be used to describe environmental fate profiles of antimalarial drugs in the environment.

TU 040

Matching micropollutant loads of influent and effluent for reliable mass balances in WWTPs
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The calculation of the effluent concentrations in a WWTP is usually evaluated by data from long-term time series where variation caused by transient flow and concentration are averaged out. The analyses of micropollutants is however work and cost-intensive and that is why measurement campaigns usually only cover short periods with a small number of samples available to establish a mass balance. Considering that biological reactor tanks are well-mixed systems, a sampling scheme comprising single 24-h composite samples was used to compare calculated and measured concentrations in WWTP effluents. For a completely-mixed system, it was shown that a 24-h effluent load is composed of water volume fractions from several previous days (Majewsky et al., 2011).

In the present study, we designed an individual sampling strategy using hydraulic calibration of a Luxembourg WWTP to validate the proposed model. The elimination efficiencies of selected pharmaceuticals were calculated based on the basis of 24-h composite samples (time-proportional) which allowed to explain >80% of the origin of the effluent load sampled. Results showed that the method was capable of reproducing realistic elimination values while short-term mass balances lead to erroneous results. The model allowed that to compare the accuracy of different sampling scenarios as a function of the sampling mode and frequency (Ort et al., 2010) and of the number of samples taken in the inlet and outlet of the WWTP. The aim is to maximally reduce the uncertainty and/or to explain the bulk of the effluent load origin. A relationship can be established between the influencing parameters that are captured by an efficient sampling period and the hydraulic retention time (HRT). Based on these findings, we propose to develop a generic design method for sampling strategies in WWTPs without having to rely on prior hydraulic calibration.


Ort, C., Lawrence, M.G., Rieckermann, J., Joss, A., 2010b. Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your campaigns valid? A critical review. Environmental Science & Technology 44 (16), 6024-6035.

TU 041

Occurrence and fate of triclosan and triclocarban during wastewater and biosolid treatment
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Triclosan (TCS) and triclocarban (TCC) are broad spectrum antimicrobial agents extensively used in a wide variety of personal care products including antibacterial soaps and detergents, shampoos, deodorants, cosmetics, mouth rinses and toothpastes.

During wastewater treatment, the removal efficiency of triclosan and triclocarban from the aqueous phase can be considerable in activated sludge processes but is dependent on temperature and pH conditions. Triclocarban is recalcitrant during the sewage treatment process and is detected in streams and sediments downstream of WWTPs.

A problem with these triclocarbons is that they are often produced in processes intended for land application. Thus, the agricultural recycling of biosolids, the favored option for sludge management, may lead to adverse environmental impact.

In this work, TCS, TCC and MTS were monitored in both waters and sludge at different stages within the wastewater treatment process in two WWTPs with different characteristics located in the coastal area of Girona (NE Spain). Moreover, the influence of sludge digestion process on the degradation of target compounds was evaluated by analyzing the resulting digested biosolids under both aerobic and anaerobic conditions. The effect of the temperature on the occurrence of the studied compounds in the final biosol was also investigated.

TU 042

Dominant time scale for antibiotics concentrations in rivers through a one-year field campaign study
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Among the long list of pharmaceuticals that can be found in the aquatic environment, antibiotics receive important and increasing attention, as they are suspected to foster bacterial resistance. But if many studies have listed their constant presence in waste waters, few of them curiously have focused on the temporal dynamics of their concentrations. In the presented study, we compare the relative importance of monthly, weekly, daily, and hourly time scale when assessing the dynamics of antibiotics in waste waters. For this, a one-year experimental sampling campaign was conducted at the Vidy WWTP of Lausanne in Switzerland. One week a month during one year, hourly samples were taken from WWTP influent. Samples were aggregated together to obtain measured hourly variations, daily variations, monthly variations and seasonal variations. A total of more than 180 samples over year 2011 were analyzed using UPLC-MS/MS, and the concentrations of 9 antibiotics (azithromycin, ciprofloxacin, ofloxcacin, clarithromycin, clindamycin, metronidazol, norfloxacin, sulfamethoxazol, trimetoprim) compared. We conclude that even if a seasonal trend for concentrations can be observed for several substances, the hourly time scale totally dominates temporal fluctuations of concentrations. In other words, there is a high hourly fluctuation of antibiotics concentrations, which is superior in magnitude to other (daily or seasonal) time scales. Thus, more efforts in terms of modelling and treatment optimization should be conducted at this temporal resolution (hour) for antibiotics, in order to reduce the risk they can represent for the environment.

TU 043

Quantification of pharmaceuticals and endocrine disruptors in river sediments: development and validation of a QuEChERS based extraction
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Accessing exposure concentrations can be challenging for the analyst as environmental concentrations and toxicological or eco-toxicological effect induced quantities are often limited by the sample capacities. In worst case scenarios, limited sample concentration and purification steps like Accelerated Solvent Extraction (ASE) and Solid Phase Extraction (SPE) to extract and analyse pharmaceuticals or hormones at the lower or sub ng/g level from solid matrices. A recent extraction method known as Quick Easy Cheap Effective Rugged and Safe has been developed for the analysis of pesticides in food matrices. This method is called QuEChERS extraction and was developed to handle complex and demanding mixtures.

In this study, we applied the QuEChERS extraction to the screening of multi-families of pharmaceuticals and endocrine disruptors in river sediments. The molecules were chosen among the priority pollutants listed in a prioritisation for French surface waters [2], in the Water Framework Directive and within the potential or confirmed EDCs. This selection of 13 pharmaceuticals includes β-blockers, anti-inflammatories, antibiotics, androgens, anti-depressants and anti-genes 24 EDGs includes pesticides, alkylphenols, hormones and phenolic derivates and. Quantification is performed on a 3200QTrap tandem mass spectrometer (MS/MS) coupled to Liquid Chromatography (LC) Agilent 1200 system. We used a method for the extraction of the compounds in the form of solid-phase extraction (SPE) and SPE coupled with High Performance Liquid Chromatography (HPLC) with a Waters Acquity UPLC `

In this work, TCS, TCC and MTS were monitored in both waters and sludge at different stages within the wastewater treatment process in two WWTPs with different characteristics located in the coastal area of Girona (NE Spain). Moreover, the influence of sludge digestion process on the degradation of target compounds was evaluated by analyzing the resulting digested biosolids under both aerobic and anaerobic conditions. The effect of the temperature on the occurrence of the studied compounds in the final biosol was also investigated.

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References

TU 044

Developing a miniaturized push-pull test to study the transformation of pharmaceuticals in the hyporheic zone of rivers
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The hyporheic zone is a key compartment for the attenuation of organic micropollutants in rivers. The comparatively slow flow velocity, the continuous import of nutrients and oxygen, and the export of metabolic waste provide ideal conditions for microbial transformation processes. A better characterization of hyporheic processes would substantially improve the quantification of the self-purification capacity of streams. Unfortunately, measuring transformation rates of organic micropollutants directly in the hyporheic zone is difficult due to limited sample capacities and also due to difficulties in experimental methods. Therefore, we are developing a miniaturized push-pull methodology that allows measuring transformation rates of pharmaceuticals directly in the hyporheic zone.

Push-pull tests are an established method of testing the transformation capacity of aquifers with respect to organic or inorganic contaminants. There, a column containing a set of conservative and reactive tracers is injected (“push”) into an aquifer, and after a certain time the groundwater is extracted (“pull”) through the same well. The difference between conservative and reactive tracer recovery can then be used to estimate in-situ transformation rates. The aim of our study is to downscale this approach so that it can

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be used on the spatial scale of centimetres to decimetres. The experimental concept is validated in a small flume that allows the simulation of different hydraulic conditions in the hyporheic zone. Under abiotic conditions, we applied the approach successfully to measure retention of several pharmaceuticals at residence times of up to 16 hours; longer residence times will be evaluated. Currently, we are experimenting with the approach to estimating the aquatic bioconcentration factor using reduced sampling has been developed (Springer, et al. 2008). This reduced sampling approach aims to screen for water-sediment transformations in a laboratory test and compares those to what may be predicted in an expert system to understand how selective the predictions may be and what additional information might be helpful in making a predicted pathway more realistic. Challenges in screening for water-sediment transformations in a laboratory test are also discussed to assess how such a test might be performed. For both situations, case studies will be presented to highlight some of the challenges present in advancing these further.

TU 046 Water-sediment biodegradation: challenges in modeling and screening for pharmaceutical transformation products

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The current OECD 308 test is a water-sediment simulation test that is typically conducted over a 100-200 day period. It is intended to estimate the parent half life in the water phase, sediment phase and collectively in the total water-sediment system; determine the distribution and mass balance of the residues in the test system; and as close as possible to the transformation phase of the parent compound. The OECD 308 test period is not prolonged. Given the long exposure time over the study period, the OECD 308 test is an expensive and costly associated with conducting the test, it is often desired that a more rapid screen for assessing the potential transformation of pharmaceuticals would be available. Such information would be helpful in developing the ERA testing strategy especially when the identification of a key transformation product would be helpful early on in the risk assessment screening sampling intervals. To address these concerns, we present an evaluation of the OECD 308 study and compares those to what may be predicted in an expert system to understand how selective the predictions may be and what additional information might be helpful in making a predicted pathway more realistic. Challenges in screening for water-sediment transformations in a laboratory test are also discussed to assess how such a test might be performed. For both situations, case studies will be presented to highlight some of the challenges present in advancing these further.

TU 047 A comparison of fish bioconcentration factors for several pharmaceutical compounds obtained following the standard OECD 305 Protocol and the estimated BCFs obtained using the reduced sampling method

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Understanding whether an active pharmaceutical ingredient is likely to be taken up from the aquatic environment by fish and bioaccumulate has triggered the need to conduct bioconcentration studies in fish. Such studies are typically considered appropriate for pharmaceuticals having a logD value > 3 and required, as per the EMA Guideline, for pharmaceuticals with logD values > 4.5.

The standard OECD Guideline 305 includes an exposure (uptake) phase followed by a post-exposure (depuration) phase typically equal to the duration of the uptake phase. During the uptake phase, between 14 and 60 days, water and fish samples are analyzed periodically until steady state has been established and the depuration phase may be initiated. During the depuration phase, analysis of water and fish samples continues until a plateau has been reached. Based on the sampling schedule outlined in the guideline, 100 fish per study may be required to determine a kinetic bioconcentration factor (BCF). In an effort to reduce the number of fish required to obtain a BCF, an approach to estimating the aquatic bioconcentration factor using reduced sampling has been developed (Springer, et al. 2008). This reduced sampling approach aims to estimate BCFs using a minimum number of sampling time points and therefore a reduced number of fish. The goal of this project is compare the BCFs of several active pharmaceuticals obtained through standard OECD 305 test methods with those BCFs determined using the approach of estimating aquatic bioconcentration factors using the reduced sampling method.

TU 048 Waterborne beclomethasone dipropionate affects fish while beclomethasone is not taken up

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Glucocorticoids are steroid hormones, playing important roles in several aspects of vertebrate physiology. The synthetic corticosteroid beclomethasone has anti-inflammatory activity, as well as modest effects on lipid metabolism and glucose levels in fish. Although levels of beclomethasone and its lipid esters in effluents or surface waters are not known, it can be assumed that most of the consumed product BPM is metabolized into the lipophilic forms before reaching the environment. The synthetic corticosteroid beclomethasone has anti-inflammatory activity, as well as modest effects on glucose levels in fish. Although levels of beclomethasone and its lipid esters in effluents or surface waters are not known, it can be assumed that most of the consumed product BPM is metabolized into the lipophilic forms before reaching the environment. We have therefore compared the potential of beclomethasone and BPM to achieve the physiology of exposed fish. Controlled flow-through studies with rainbow trout over two weeks up to or 0.65 µg of beclomethasone/L did not result in measurable plasma levels. Exposure to 0.65 µg/L BPM, on the other hand, led to accumulation of both beclomethasone, BMP and BDP in plasma, indicating a considerably more efficient uptake of the more lipophilic prodrug as well as metabolism within the fish. Accordingly, exposure to 0.65 µg/L of BDP significantly increased blood glucose levels, in agreement with previously published results. Furthermore, we could demonstrate a parallel increase in catalase activity in liver tissue, while exposure to beclomethasone had no effect on these endpoints. We are currently screening for beclomethasone, BPM and BMP in sewer effluents and surface water.

TU 049 The degradation of antidepressant pharmaceuticals in aerobic wastewater treatment

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The antidepressant pharmaceuticals sertraline and venlafaxine are common contaminants in both aquatic and terrestrial environments and are reported to produce adverse effects on a range of aquatic organisms. A key point of entry of pharmaceuticals into the environment is through wastewater treatment. To learn whether microbial communities in wastewater sludge have the ability to degrade sertraline and venlafaxine and, thus, consequently reduce their environmental loads, microcosms containing aerobic wastewater were spiked with sertraline and venlafaxine. Samples collected over 36 days were analyzed for the depletion of the added pharmaceuticals by high performance liquid chromatography tandem mass spectrometry (LC/MS/MS). Municipal aerobic wastewater sludge was found to degrade sertraline and venlafaxine by over 90% over the course of 36 days, whereas no change in the added sertraline and venlafaxine concentrations were observed in abiotic control microcosms.

TU 050 Time trend of cyclic volatile methyl siloxanes in Baltic herring

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Cyclic volatile methyl siloxanes (CVMs) were measured in Baltic herring (Clupea harengus) from the Swedish environmental sample bank. Fish from a site from the Baltic Proper southeast of Stockholm (Landset) and from the northern part of the Bothnian Bay (Harutjärn) were analysed. Skinless dorsal muscle samples from six individuals were pooled. Two pooled samples were analysed for each sample year from the period 1989 - 2009. Octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6) were analysed. The D5 concentrations were of the 50 ng/g lipid weight, while the concentrations of D4 and D6 were lower and frequently below detection limits. The concentrations were generally lower at the beginning of the period and highest around 2005-2007. There were no marked differences in concentration in the two locations.

TU 051 Degradation studies of the ionophores lasalocid, monensin, narasin, and salinomycin

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The ionophores lasalocid, monensin, narasin, and salinomycin are commonly used antimicrobial agents in animal feed. These ionophores exhibit a wide range of toxicities in aquatic environments, and their degradation in wastewater treatment is of particular concern. A study was conducted to assess the degradation of these ionophores in a municipal wastewater treatment plant. The results indicated that lasalocid was degraded to a significant extent, while monensin, narasin, and salinomycin were not degraded to a notable degree. These findings have implications for the management of wastewater treatment plants and their impact on the aquatic environment.

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Sorption process of three sulfonylurea based of anti-diabetic drugs were tested on three various soil. Selected soils differed in their physical and chemical properties and thus, drugs, suggesting the possibility of other sources flowing into the water environment. Overall, anthelmintics showed insignificant removal efficiencies in wastewater treatment and therefore be useful for studying pharmaceutical transfer and fate in the coastal environment.

Antibiotics are micropollutants which release in the aquatic environment has been a subject of concern for several years as they can give rise to two problems: i) they can have effects on the natural environment, ii) they can promote the development of resistance to antibiotics and the transfer of antibiotic resistance genes between bacterial species, including those affecting human health.

Degradation of antibiotics by photocatalysis on immobilized titanium dioxide. The aim of this study is to describe the environmental fate of ionophores and to identify new and ecotoxicologically relevant transformation products. The formation and presence of such stable transformation products in the effluent of sewage works, surface water, and drinking water treatment is reported in the scientific literature with increasing frequency. In only a few cases, full mineralization of the parent compounds is achieved. This is even more of importance as advanced oxidation processes employing e.g. ozone, hydrogen peroxide, light or electro-coagulation are increasingly under discussion for the removal of pharmaceuticals and other micro pollutants in effluent treatment and drinking water treatment. Treatments using these techniques may even lead to the formation of transformation products that are more toxic than the parent compound.

For a selection of pharmaceuticals of both classes, the formation of stable transformation products in various stages of the water cycle (both drinking and sewage) will be investigated in laboratory and pilot scale studies. Treatment processes include mainly chlorination, ozonation and UV disinfection for drinking water treatment and advanced oxidation processes for pharmaceutical waste. Treatment processes for structure elucidation of the transformation products formed, different LC/MS/MS approaches as well as high resolution MS techniques will be implemented. Characterization of the stable transformation products in terms of (eco)toxicological behavior will be done using a battery of in vitro bioassays.

Predicting environmental concentrations of carbamazepine and oxcarbazepine and their main metabolites in a coastal system. Degradation of antibiotics by photocatalysis on immobilized titanium dioxide. The aim of this study is to describe the environmental fate of ionophores and to identify new and ecotoxicologically relevant transformation products. The formation and presence of such stable transformation products in the effluent of sewage works, surface water, and drinking water treatment is reported in the scientific literature with increasing frequency. In only a few cases, full mineralization of the parent compounds is achieved. This is even more of importance as advanced oxidation processes employing e.g. ozone, hydrogen peroxide, light or electro-coagulation are increasingly under discussion for the removal of pharmaceuticals and other micro pollutants in effluent treatment and drinking water treatment. Treatments using these techniques may even lead to the formation of transformation products that are more toxic than the parent compound.

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of photodegradation of two antibiotics often observed in the environment: a veterinary drug, tylosin (916 g/mole, pKa = 7.1) and a human drug sulfamethoxazole (253 g/mole, pKa = 5.7). Two photocatalysts (titanium dioxide) have been used: PC500 (Millenium) and P25 (Degussa). Two modes of immobilization have been tested: on a glass plate in the lab for both photocatalysts or on cellulose fibers for PC500 only (Ahlstrom, Pont-Evêque, France). Antibiotics degradation was monitored by UV-visible spectrometry and GC-MS. End products (organic carbon and nitrogen species) were also quantified. Degradation was observed, P25 being more efficient than PC500. Besides the mode of immobilization, other parameters have been investigated such as pH, position of the lamp, flow rate and antibiotic initial concentration.

TU 057

Direct and indirect photolysis of human metabolites of antibiotic sulfamethoxazole

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Both direct and indirect photodegradation processes have been reported to be important removal mechanisms of sulfamethoxazole (SMX) in the environment. However, most of SMX and its metabolites are hydrophilic, yet as their environmental fate is not known. In this study, we assessed the direct and indirect photodegradation of three human SMX metabolites: 3-hydroxy-SMX, 4-aminobenzyl-SMX, and 3-aminobenzyl-SMX in artificial seawater at 3 different pHs of 5, 7, and 9. The photodegradation rates of all three metabolites were observed to be environmentally relevant pH 8.4, where the anionic form of the compounds predominates, except for SMX-glucuronide. The faster degradation of the neutral basic drug was attributed to SMX-glucuronide missing an acidic functionality, therefore, in contrast to SMX and other metabolites, it remains in its neutral and more photoreactive form at ambient pH. The contribution of indirect photolysis to the overall photolysis was found to be greatest for nitro-SMX (70% in presence of 10 mg/L of nitro-SMX). Experiments conducted in lake water (1.19 mg/L NO3−, 2.43 mg/L TOC) showed degradation rates comparable to humic acid experiments. Furthermore, we examined the occurrence of SMX and metabolites in the proximity of a wastewater discharge in Lake Geneva, Switzerland. Two human metabolites of SMX, acetyl-SMX and SMX-glucuronide were regularly detected in wastewater effluent and lake water samples, in concentrations lower than that of the parent compound.
A comprehensive evaluation of the effects to the antimicrobial personal care product triclosan (TCS) in the terrestrial environment was performed. This evaluation included the effect on TCS soil metabolism, microbial respiration and microbial respiration inhibition on terrestrial arthropods and woodcutters, effects to earthworms (Eisenia fetida), and effects on emergence and growth terrestrial plant species. Microbial respiration and nitrification were evaluated in soil treated with 0.1-2.0 mg/Kg TCS for 28 days. The acute and chronic toxicity of TCS to worms were tested in an artificial soil in accordance with OECD guidelines 207 and 222. Test concentrations for acute and chronic toxicity studies were chosen from 64-100 and 14-100, respectively, for soil respiration studies, respectively. The effects of TCS on the predatory mite Hypoaspis aculeifer exposed to 0.3-320 mg/Kg (dw) TCS for 14 days were evaluated in accordance with OECD guideline 226. Phytopathogenic studies evaluating survival, emergence, biomass, and length, and normality of development in 10 species of terrestrial plants were performed in accordance with OECD guideline 208 using 0.2-1,000 mg/Kg (dw) TCS. Survivors were exposed for the median emergence time plus 14 days. No Observed Effects Concentrations (NOEC) and Lowest Observed Effects Concentrations (LOEC) for soil respiration and soil nitrification were 2 and 2 mg/Kg (dw), respectively. NOEC and LOEC values for acute and chronic toxicity to earthworms were 1,026 and >1,026 mg/Kg (dw) [survival], and 100 and >100 mg/Kg (dw) [survival and reproduction], respectively. NOEC and Lowest Observed Effects Concentrations (LOEC) values for root emergence and reproduction were 10 and 12 and 32 and 4 mg/Kg (dw), respectively. The LOEC values in terrestrial plants ranged from 75 and 100 mg/Kg (buster in lettuce) to 1,000 and >1,000 mg/Kg [emergence and phytotoxicity in wheat and phytoxcity in cucumber]. Moreover, these studies demonstrate that TCS poses minimal risk to the terrestrial environment which is consistent with Reiss et al. (2002).

TU 066

Risk assessment of pharmaceuticals incidentally discharged to the terrestrial environment


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The Environmental Risk Assessment (ERA) of pharmaceuticals mainly considers the aquatic compartment, since the majority of active pharmaceutical ingredients (APIs) are hydrophilic. However, with more hydrophobic APIs, much more remains in sewage sludge during municipal sewage treatment, and following application of sludge to land as fertilizer. There is a potential for chemical residues in soils. With the rapid growth of the human population, water use, re-use practices are diverse and increasingly global. The irrigation of crops with raw or treated wastewater could act as a potential source of APIs to the food chain. Exposure via this route and its significance is not currently considered within ERA guidelines. The disposal of unused medicines is also of interest, and recent monitoring studies have shown that drugs disposed to landfill can potentially leach out and contaminate local groundwater and surface water. This poster presentation describes a risk assessment for pharmaceuticals associated with emission potential routes to the terrestrial environment: application of biosolids to agricultural land, sludge applied to natural land; unused medicines to landfill. The potential risks have been evaluated with modelling techniques contained within the European Commission's technical guidelines, and where input parameters are not available, estimation methods have been adopted. The assessment highlights the relative risk between each of these emission routes and how uncertainty in key parameters can affect the outcome. This indicates the potential need for additional pharmaceutical fate study data in order to carry out robust risk assessments associated with the terrestrial environment.

TU 067

The ScenAT exposure model: a novel spatial method to inform environmental risk assessments of personal care products in China

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The China economy has grown significantly and so has the demand for personal and household care (PHC) products. The detection of chemicals used in PHC products is increasing in profile as China develops models for use in prediction of environmental concentrations (PEC). Data required to accurately determine PEC's of PHC chemicals include product usage information, physico-chemical properties, environmental behaviour (e.g. persistence, adsorption) and use of environmental information (e.g. receiving water bodies, sewage treatment plant infrastructure) which form the basis for exposure estimation models allowing PECs to be generated at a more spatially resolved level (e.g., 2769 counties in China). Per capita water use values were sourced at the provincial level and the population connected to STP's were calculated using per capita water use and the STP domestic consumption discharge. Dilution factors were calculated per country using the population, influencing area and waste water flow. Predicted environmental concentrations were determined using the fraction removed in STP's in the domestic water use per capita, the annual tonnage of ingredient used, the population dilution factors in receiving waters. The highest PEC's can be seen in North and East China. These areas have increased water scarcity and contain large densely populated cities like Beijing and Shanghai that will contribute to the increased concentrations. Model validation is difficult to perform in real time due to the long term measurement of the entire area of Lombardy. Therefore a qualitative assessment was performed to determine if spatial patterns of water quality were consistent with data published by the Ministry of Environmental Protection. The results show the PECs are qualitatively consistent with other assessments of water quality. The model fits neatly between a unit world approach (e.g. EUSES, RAIDAR and USEtox), which are quick and easy to run and catchment models (e.g. GREETER and PHate) which give a better approximation of real world but are complex and data heavy.

TU 068

A concordance assessment of Predicted-No-Effect-Concentration (PNEC) aquatic toxicity data for pharmaceuticals

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Environmental safety assessments are required for market approval of new Active Pharmaceutical Ingredients (APIs) (in the EU and to a lesser extent in the US), however, such assessments may be lacking for old drugs. To determine whether available environmental toxicity data can be used to make PNEC predictions for APIs for which effect data are lacking, we analyzed publicly available data for 197 APIs. The APIs included in the Stockholm County Council booklet, Environmentally Classified Pharmaceutical (ECP), 2011 Edition were compared with the APIs used in the analysis of the booklet in the case of a PNEC generated (195), and an additional two, the supporting data were extracted from the Swedish National Formulary website (www.FASS.se) and parsed to facilitate statistical treatment. Following an outlier analysis, the dataset was used to evaluate statistical correlations between the PNEC and trophic level (algae, fish, invertebrate), benchmark type (EC50/NOEC), assessment factor, and Anatomical Therapeutic Chemical (ATC) Classification System group. Based on the findings of the statistical analysis, we consider two potential approaches for generating PNECs for APIs for which no effect data are available, discuss their strengths and weaknesses and future development needs.

TU 069

Environmental risk assessment for the polycyclic musk AHTN and HHCB in the Molgora river (Lombardia Region, Italy)

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The Molgora river is one of the most important Italian rivers flowing from the Alps to the Po Valley and entering into the Adriatic Sea, near Venice. The river was chosen for the study because it is capable of hosting a large number of natural and artificial species, in addition to alluvial and urbanizing waters. The river flows through the Lombardia Province of Northern Italy, which is characterized by an urbanized river and has increased water scarcity and contains larger densely populated cities like Milan and Turin. Therefore a qualitative assessment was performed to determine if spatial patterns of water quality are consistent with data published by the Ministry of Environmental Protection. The results show the PECs are qualitatively consistent with other assessments of water quality. The model fits neatly between a unit world approach (e.g. EUSES, RAIDAR and USEtox), which are quick and easy to run and catchment models (e.g. GREETER and PHate) which give a better approximation of real world but are complex and data heavy.

TU 070

Does price pressure on medicines result in more pollution?

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Pharmaceutical industries are manufacturing more and more of their polluting the environment with high concentrations of a variety of APIs, including broad-spectrum antibiotics. Additionally, these compounds are often produced in soft-boundaries, which of the world suggest that this is not an isolated problem. However, information on by whom, where and under what circumstances the API of a given pharmaceutical product is produced is not publically available. Effectively prevents prescription and consumers to make environmentally informed decisions, and thus reduces incentives for API manufacturers to invest in green production technologies. The Swedish Medical Products Agency has information on where and by whom the API is produced for products approved for the Swedish market, data which can be accessible for research purposes. A smaller, previous study of our research group, exploring the API's of 31% for 342 investigated products sent their waste to a treatment plant in Patancheru with insufficient waste water treatment. This disturbingly high figure can however not be extrapolated to the entire Swedish market since we preselected 9 APIs known to be present in the effluent of the Indian market. Also, various sales figures were not accounted for. The aims of the present study is to determine where the APIs in pharmaceutical products, sold in Sweden, are produced and to what extent the prices of interchangeable drugs correlate to the country of origin of the API. We will also investigate if price correlates to environmental performance indexes in the manufacturing plant. Such surrogate measures are used as conclusive information on pollution level is lacking for most
Do pharmaceuticals with evolutionary preserved drug-targets pose a greater environmental risk? S.M. Furuhagen, A. Fuchs, E. Lundström, E. Gorokhova, M. Breitholtz
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Human pharmaceuticals are designed to target specific molecules involved in a particular metabolic or signaling pathway in humans to obtain desirable effects at low doses. Although not all of these drug-targets are present within the diversity of biological life found in nature, some are evolutionary well-preserved. It has therefore been hypothesized that non-target organisms with highly conserved human drug-targets may be at a greater risk to be affected by pharmaceutical residues in the environment. In this study, we investigated the hypothesis that pharmaceuticals with well-preserved drug-targets in the cladoceran Daphnia magna have greater effects on gene expression and RNA content. Three different pharmaceuticals were tested: the anti-fungal drug miconazole, the anti-histamine promethazine (both with highly conserved drug-targets in D. magna), and the progestogen levonorgestrel (with a low drug-target homology). For each pharmaceutical, juvenile daphnids were exposed to sublethal concentrations and raised to instar 3. The effects were assayed at the level of (1) gene expression (vitellogenin and cuticle protein mRNA using magna), and the progestogen levonorgestrel (with a low drug-target homology). For each pharmaceutical, juvenile daphnids were exposed to sublethal concentrations and raised to instar 3. The effects were assayed at the level of (1) gene expression (vitellogenin and cuticle protein mRNA using magna), and (2) relationship between total RNA and DNA (as a proxy for protein synthesis rate), and (3) body size. The exposure to miconazole resulted in a two- and three-fold decreased gene expression of vitellogenin and cuticle protein genes, respectively, as well as a significant increase in RNA content in relation to DNA. The down-regulated expressions of vitellogenin and cuticle protein genes are indicative of estrogenic mRNA that can be a result of enhanced protein synthesis as a factor to stress. By contrast, promethazine and levonorgestrel did not alter RNA-DNA relationship. No effects on the body size were observed for either test substances. Thus, our preliminary results indicate that for miconazole, the drug with well-conserved drug-targets, the endocrine and protein synthesis disrupting effects were indeed observed at sublethal concentrations. To fully test the hypothesis, complementary analyses on the other two pharmaceuticals are also performed.

TU 074 Transcriptome analysis of the brain of the gilthead sea bream (Sparus aurata) after exposure to environmentally relevant concentrations of human pharmaceuticals M. Hampe1, M. Milan, J. Blasco, S. Ferraresi2, L. Bargelloni1
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Pharmaceuticals are being released into the environment in extremely large quantities on a regular basis. Ingested drugs are eventually excreted from individuals, as well as they are released from manufacturing and disposal of unused or expired drugs ultimately winding up in the effluent of wastewater treatment plants and aquatic environments. In order to assess the long term effects of exposure environmentally relevant concentrations of representative pharmaceutical compounds we have exposed the gilthead sea bream (Sparus aurata) to representative drugs found at ng-μg L-1 levels in surface- ground and coastal waters. Acetaminophen (APAP), Carbamazepine (CBZ) and Atenolol (AT) were used as model compounds to assess the treatment with the greatest number of differentially expressed genes was CBZ with 467 features, compared with 226 for APAP and 4 for AT. The selected vehicle, DSMO, didn’t show any significant expression changes in comparison with the SW control. Out of all these features, only one was common between all the three treatments. This feature was identified by homology search as Sparus aurata cytochrome c oxidase subunit I mRNA. No other feature was common both between APAP and AT nor CBZ and AT. However, there were 130 common features between the APAP and CBZ treatment.

Functional annotation clustering and enrichment analysis of APAP and CBZ candidate genes in DAVID revealed treatment specific activation of different pathways and processes, with energy-related features present in all the applied treatments.

TU 075 Chronic effect of atenolol on physiological indices in rainbow trout (Oncorhynchus mykiss) V. Zlabek, V. Burkina, H. Kroupova, C. Steinbach, R. Grabic, G. Fedorova, J. Velisek, T. Randak
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Atenolol is a representative of second generation of the beta-blockers, which is often detected in the surface water, due to its extensive use and relatively high stability. In this study, we investigated the effects of atenolol on all standard indices of juvenile rainbow trout, Oncorhynchus mykiss, by chronic semi-static bioassay. Fish were exposed to sublithral concentrations of atenolol (1, 10 and 1000 μg/L) for 42 days. Multiple biomarkers were measured, including morphologic indices, haematological parameters, antioxidant responses and CYP450 activities. Parameters measured in this study displayed various dependent patterns to atenolol concentrations and exposure time. In short, the multiple responses in fish indicated that atenolol induced physiological stress and could be used as potential biomarkers for monitoring residual atenolol in aquatic environment.

Molecular and genetic mechanisms of these physiological responses in fish are not clear and need to be further studied.

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TU 076 Investigating the genotoxicity effects of pharmaceutical photo-transformation products M.L. Vasquez1, M.I. Garcia-Käufer2, E. Hapeshi3, K. Kümmerer2, D. Fatta-Kassinos1
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All types of urban wastewater discharge and reuse practices, including irrigation of landscape and agricultural areas, groundwater replenishment, discharge into inland surface waters and sea, cause the release of organic xenobiotic substances in the environment. Amongst them, pharmaceuticals, which are widespread-used and dissimilar chemical compounds, specifically designed to interact with living organisms, represent an important concern for humans and the ecosystems. It has already been proved that conventional treatment taking place in urban wastewater treatment plants is incapable of eliminating these substances and as a result pharmaceuticals, their metabolites and/or transformation products are released in the environment. Research has been concentrated mainly on the effects the parent compounds may have; whereas investigation of possible effects caused by metabolites and transformation products is still at its early stage.

This study has been focused on investigating effects of photo-transformation products of oxiloxan, a photolabile second generation fluoroquinolone, widely used and frequently found in the environment. Photo-transformation of oxiloxan was achieved by photonic and photocatalytic treatment at various times. Nine photo-transformation products and degradation processes that based on piperazinyl decyclization and decarboxylation are proposed. Furthermore, their genotoxicity using the cytokinesis-block micronucleus assay with the hepatocellular liver carcinoma cell line (HePG2) was studied and demonstrated that a long irradiation time (greater than 30 min) is required to eliminate possible genotoxic effects.

TU 077 Ecotoxicity of 14 serotoninergic pharmaceuticals in the crustacean Daphnia magna, the microalga Pseudokirchneriella subcapitata and the marine gastropod Haliotis tuberculata E. Farcy1, R. Bureau2, A. Serpentini3, J.M. Lebel1, M.P. Halm4
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4The serotonin, also named 5-hydroxytryptamine (5-HT), acts both as a neurotransmitter or as an hormone, depending on its localization. The presented work investigated the ecotoxicological effects of 14 pharmaceuticals having serotonergic properties, i.e. enhancing the effects mediated by serotonin in the central nervous system. The tested

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molecules are mainly antidepressants (fluoxetine, sertraline, paroxetine, fluvoxamine, citalopram, clomipramine, amitriptyline, mianserin, milnacipran, duloxetine, venlafaxine) but also antipsychotic drugs (clozapine), cough suppressant/hallucinogen drug (dextromethorphan) or beta-blocker (propranolol). These molecules have the common property to play directly or indirectly a role in the serotonin transporter and/or serotonin receptors. The 14 molecules were tested for their ecotoxicological effects using chronic and acute bioassays on the microalgae *Pseudokirchneriella subcapitata* and the crustacean *Daphnia magna*. Additionally to these conventional bioassays, a novel bioassay was developed using primary cell culture of hemocytes from the marine gastropod abalone (*Halostis tuberculata*). This assay was used in order to address the question of pharmaceutical effects in a marine species. The acquired data were used to build quantitative structure activity relationship (QSAR) modeling in order to determine if the chemical properties of the molecules can explain their toxicity, (2) identify the mode of action of the selected pharmaceuticals.

TU 076

**Environmental effects of anticholinesterasic therapeutic drugs on a crustacean species, *Daphnia magna***

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The environmental presence of pharmaceutical drugs is nowadays an important field of toxicology, since the presence of such residues can cause a series of deleterious effects on exposed biota. This study assessed the ecotoxicological effects of two anticholinesterasic therapeutic drugs, neostigmine and pyridostigmine in *Daphnia magna*. This evaluation was conducted on an immobilization and feeding inhibition (response) and on the concentration of the two compounds. The pharmacological effects of neostigmine and pyridostigmine derive from their capacity to induce a reversible inhibition of cholinesterase activity, and have thus been used to test the human disease myasthenia gravis. We observed alterations at a sub-individual parameter directly related to the anticholinesterasic activity. Our study calculated 48 h-EC	extsubscript{50} values in the immobilization assay of 167.7 μg L	extsuperscript{-1} for neostigmine, and 44.7 μg L	extsuperscript{-1} for pyridostigmine. In the reproduction assay, the most affected parameter was the somatic growth rate (LOECs of 21.0 μg L	extsuperscript{-1} and 2.9 μg L	extsuperscript{-1} for neostigmine and pyridostigmine, respectively), followed by the fecundity (LOECs of 41.9 μg L	extsuperscript{-1} and 11.4 μg L	extsuperscript{-1} for neostigmine and pyridostigmine, respectively). We also determined a 48 h-EC	extsubscript{50} for cholinesterase activity of 1.7 and 4.5 μg L	extsuperscript{-1} for neostigmine and pyridostigmine, respectively. These results demonstrate that both compounds are extremely toxic for *D. magna* at concentrations of the order of μg L	extsuperscript{-1}. By comparing the here-obtained results with the actual concentrations of pyridostigmine previously reported in the aquatic environment (0.22 μg L	extsuperscript{-1}), it is possible that this compound can significantly alter the feeding behavior of *D. magna*. Hence, the ecological implications of these data are of great importance and must be considered under the risk assessment framework of both pharmaceutical drugs.

TU 079

**On-going chronic tests will determine whether the side effects reported during acute tests are observed**

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Drugs administered in man are eliminated through the excreta in either an intact or metabolized form. As other drugs, chemotherapeutic drug residues were measured in wastewater and in tissue samples. They reach highly polluted areas in the environment where they have the potential to persist. Consequently, their prolonged presence in surface water exposes the fauna and flora to potential adverse effects.

Tamoxifen is a synthetic non-steroidal anti-estrogenic analog inhibiting competitively estrogen receptors. This drug is mostly metabolized into metabolites such as Endoxifen and 4-hydroxy-tamoxifen (4-OH-Tam). It was recently discovered that the pharmacological activity of those two metabolites are more potent than the original molecule in vitro. As no literature exists on acute and chronic effects of these metabolites on aquatic organisms, *Daphnia pulex* have been exposed to Tamoxifen and its two metabolites in acute and chronic experimental designs. In acute tests, the concentrations that induced an effect on 50% of the tested organisms (EC50) were quite similar for Tamoxifen and 4-OH-Tam, i.e. 0.52 and 0.60 μg mL	extsuperscript{-1} respectively. Even if endoxifen showed higher EC50 (1.09 μg mL	extsuperscript{-1}) signs of distress were observed on daphnids exposed to low concentrations of this molecule (0.79 μg mL	extsuperscript{-1}) as lethargy compared to the control. On-going chronic tests will determine whether the side effects reported during acute tests are observed on *Daphnia* and their offspring exposed to low drug concentrations. In these tests, Daphnids (<24h) are placed individually in increasing concentrations (between acute and sub-lethal toxicity). The viability of the neonates is also evaluated. Such tests should give a better assessment than acute tests of the potential ecotoxicological impact of Tamoxifen and its metabolites on aquatic ecosystems.

TU 080

**Calcium inhibition as a mode-of-action of antifungal imidazole pharmaceuticals in non-target organisms: implications for mixture toxicity assessment**

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Aphrodisiac activity is no addressed in current regulation of pharmaceuticals chemicals, several theoretical models have been developed and applied to predict mixture toxicity in environmental research. The Concentration addition (CA) model describes the mixture toxicity of components with similar mode of action (MOA).

Imidazoles are primarily known as antifungal substances interfering with synthesis of ergosterol in fungi. However, imidazoles are also competitive antagonists of calmodulin (CaM). CaM is a calcium binding protein expressed in all eukaryotic cells, where it participates in many signaling pathways (e.g. nitric oxide signaling), thereby affecting many different cellular functions, particularly neurosecretory exocytosis. Some antidepressants are unable to synthesize sterols relying on dietary sources, the sterol synthesis inhibition is not likely a primary mode of action. Here, we hypothesized that exposure to imidazoles disrupt CaM-dependent nitric oxide synthesis in microcrustaceans.

Further, assuming CaM-inhibition to be the primary MOA for these substances, we evaluated CA model as a tool for assessing mixture toxicity of imidazoles. We conducted a series of acute and combined effects of four imidazoles ( econazole, ketoconazole and two imidazoles not tested previously) in *Daphnia magna* and *Nitocra spinipes* ( copepoda, harpacticoida). The mixture was tested using fixed ratios of each individual substance (LC50 value). This exposure resulted in a calculated median lethal concentration (based on Toxic Units) of 0.86 (95% CI: lower 0.74; upper 1.01), which shows that the mixture displays concentration additive toxicity, lower than the CA model. In conclusion, imidazoles not tested previously are extremely toxic for *D. magna* and *N. spinipes*, and further studies are needed to support the hypothesized MOA of imidazoles for non-target organisms and to justify the application of CA model for assessment of imidazole mixture toxicity.

TU 081

**Behavioural and physiological responses to pharmaceutical exposure in Gammarus spp. and Fucus vesiculosus**

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Gammarids are foundation species of the Baltic Sea. These species inhabit the littoral community and are thereby exposed to substances released in the coastal areas. In this study both species were exposed to three concentrations of two pharmaceuticals, ibuprofen and propranolol. Both physiological and behavioural parameters were estimated to examine potential effects in the organisms. Respiration, feeding rate, activity with and without predator cues by a Multispecies Freshwater Biomonitor ([MFB](TRADEMARK)]) were estimated for Gammarus spp. and gross production to respiration ratio (GPR) and chlorophyll fluorescence for F. vesiculosus. Ibuprofen did not affect any of the measured parameters of Gammarus spp, significantly and only slightly affected the algae. The strongest effect was related to activity of Gammarus spp measured by the [MFB](TRADEMARK)], and results showed that propranolol decreased the activity. The addition of predator cues into the exposure water increased the activity in all treatments, but the gammarids could not compensate for the reduced activity caused by the pharmaceutical. The feeding rates of Gammarus spp. exposed to propranolol were more than two times higher in all concentrations compared to the control. Also the algae was more affected by propranolol measured as lowered GPR assays on the microalgae *Pseudokirchneriella subcapitata* but also indirectly on the *Daphnia* due to the increased activity of Gammarus spp. in combination with the stress responses in the algae, might cause unexpected indirect and cascade-effects which have implications on the littoral communities.

TU 082

**Acute effects of psychiatric drugs on *D. magna* under insecticide exposure**

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The rates of production, release and use of pharmaceuticals and of psychiatric drugs in particular is expected to increase in the next 10 to 50 years for several reasons, and so is the loading into the environment. In recent years, there has been a growing concern about ecotoxicological risk of these psychiatric drugs that interact with the central nervous system and induce antidepressancy and antipsychotic effects. In this work, we assessed the acute toxicity of the antidepressant Fluoxetine and the antipsychotic Carbamazepine (some of the more prescribed psychiatric drugs) in *Daphnia magna* mortality under exposure to carbaryl or fipronil. *D. magna* was exposed to binary mixtures of each pharmaceutical and an each insecticide in full factorial designs. To address mixture effects, the observed mortality was then compared to the expected effects of mixtures calculated from effects of single compounds exposures, based on existing concepts of mixture toxicity. This permits a quantification of combined effects and independent effects, and identifies effects which are specific to the mixture.

Although the risk for acute toxic effects of psychiatric drugs is unlikely, our results contribute with ecotoxicological data for risk assessment of psychiatric pharmaceuticals in the aquatic environment and are discussed in terms of possible ecological effects of environmentally relevant concentrations of these substances on wildlife. We advocate that sub-lethal toxicity of these psychiatric drugs should be assessed under relevant exposure scenarios such as co-occurrence with neurotoxic pesticides.
TU 083
Assessing the environmental hazard of mixtures of pharmaceuticals: combined acute toxicity of fluoxetine and propanolol to the crustacean Daphnia magna
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The aim of the present work is to evaluate the acute toxic response of the crustacean Daphnia magna exposed to individual pharmaceuticals and mixtures. We tested fluoxetine, a selective serotonin reuptake inhibitor widely prescribed as antidepressant, and propanolol, a non selective β-adrenergic receptor-blocking agent used to treat hypertension. Single and mixtures' tests were conducted in accordance to OECD 202 and ISO 6341 guidelines. Less than 24 h old daphnids were exposed for 48 h. Six replicate vessels with five individuals per vessel were tested at each treatment level. Single chemicals were first tested separately; estimated EC50 were 7.0 mg/L for propanolol and 7.8 mg/L for fluoxetine. Toxicity of binary mixtures was then assessed using a fixed ratio experimental design. Five concentrations (from 0.5 to 2 total units) and 5 percentages (100% to 99%) of each component in the mixture were analyzed. The Concentration Addition model and the Independent Action model were fitted to the obtained data. This model allows to calculate for each mixture ratio the mixed effect response, i.e. the toxicity of the mixture expressed as the sum of the single substances for compounds having similar mode of action. This analysis showed a significant deviation from the CA model that indicated antagonism. Concentrated tests were much higher than those detected in the environment; however these results are to be considered as a first step in an ongoing project aimed at assessing chronic ecological interactions of mixtures of pharmaceuticals.

TU 084
Assessing health status of radiotopes pilophantus exposed to caffeine, carbaamazine, ibuprofen and novobiocin using the neutral red retention assay
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Despite the fact that pharmaceuticals have been detected in the environment at the μg L-1 to ng L-1 range, it has been demonstrated they can cause adverse effects at environmentally relevant concentrations. To detect possible adverse effects of a contaminant in the environment, standardized short-term, sensitive and low-cost methods are usually applied to estimate chronic toxicity against organisms. Lysosomal membrane stability (LMS), has been successfully applied as a screening tool to determine the health status of a wide range of organisms. The feeding style and habitat of the manila clam (Raditupes pilophantus) make this species vulnerable to organic contaminants bound to water and it has been widely used in ecotoxicological studies. LMS was evaluated in clams’ haemolymph by the neutral red retention assay (NRRA). Claims were exposed in the laboratory to concentrations of caffeine (selective stimulant), carbaamazine (anticonvulsant and mood stabilizing), ibuprofen (non-steroidal anti-inflammatory and novobiocin (antibiotics) during 35 days in a synthetic 48 h renewal assay. Filtered sea water was spiked every 2 days with caffeine (0.1, 5, 15, 50 µg L-1), ibuprofen (0.1, 5, 10, 50 µg L-1), carbaamazine and novobiocin (0.1, 1, 10, 50 µg L-1). Stock solutions of pharmaceuticals were prepared in DMSO (0.001%) to ensure solubility. Test with this solvent was undertaken to ensure no solvent effect. Results showed that neutral red retention time (NRRT) measured at the end of the bioassay was significantly (p < 0.05) reduced when exposed to environmental concentration of caffeine (≤ 15 µg L-1); ibuprofen (≤ 5 µg L-1); carbaamazine (≤ 1 µg L-1); and novobiocin (≤ 10 µg L-1). Results showed dose-dependent effect of pharmaceuticals (p< 0.05); Claims analyzed after exposure to 10, 15 and 50 µg L-1 were considered to present a diminished health status (NRRT < 45 min) (p < 0.05). LMS appears to be a sensitive tool that enables evaluation of the health status of clams after exposure to concentrations of selected pharmaceuticals under laboratory conditions and could thus be used as a biomarker for pharmaceutical contamination in aquatic environments.

TU 086
Cytogenotoxic effects induced by cocaine on the freshwater bivalve Dreissena polymorpha
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In the global trend of consumption of illicit drugs has caused both social and medical problems, but also the onset of a potential new environmental hazard. After human consumption, in fact, drugs of abuse enter the aquatic system posing a potential risk for aquatic biocoenosis. Even if many studies have showed the presence of both drugs and their metabolites in freshwater in the high ng/L to low μg/L range worldwide, at present any information on their potentially harmful effects on non-target organisms is avaliable. The present study was to investigate the cyto-genotoxic effects induced by the cocaine, one of the most illicit drug in Western Countries, as well, consequently, one of the most found in the aquatic environment. Our goal was reached through the application of a biomarker battery on a classical freshwater biological model, the zebra mussel Dreissena polymorpha. The raise of genotoxic effects was investigated by the Single Cell Gel Electrophoresis (SCGE) assay, which evaluated primary DNA damages, and by the DNA diffusion assay and the micronucleus test (MN test), which investigated secondary genetic damage. The Neutral Red Retention Assay (NRRA), by evaluating the lysosomal membrane stability, was used to assess cocaine cytotoxicity. 96 h exposures to three increasing nominal concentrations of cocaine (40 ng/L, 200 ng/L and 1000 ng/L), carbamazepine and novobiocin = 10 µg L-1) . Results showed dose-dependent effect of pharmaceuticals (p< 0.05) Clams analyzed after exposure to 10, 15 and 50 µg L-1 were considered to present a diminished health status (NRRT < 45 min) (p < 0.05). LMS appears to be a sensitive tool that enables evaluation of the health status of clams after exposure to concentrations of selected pharmaceuticals under laboratory conditions and could thus be used as a biomarker for pharmaceutical contamination in aquatic environments.

TU 087
A comparison of cyto-genotoxic effects induced by some pharmaceutical and personal care products (PPCPs) on the freshwater bivalve Dreissena polymorpha
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Pharmaceutical and personal care products (PPCPs) are considered new environmental pollutants, since hundreds of these molecules are commonly revealed in the aquatic environment in the high ng/L to low μg/L range worldwide. Among them, the occurrence of the antibacterial triclosan (TCS), the antibiotic trimethoprim (TMP) and the non-steroidal anti-inflammatory drugs (NSAIDs) ibuprofen (IBU), diclofenac (DCF) and paracetamol (PCM) is well documented both in surface and sewage waters. Notwithstanding the potential hazards these compounds may have for target organisms. In order to enlarge this topic, sub-lethal effects induced by these therapeutic agents were investigated by using a multi-biomarker battery the freshwater bivalve Dreissena polymorpha. According to a semi-static in vivo approach, zebra mussels were exposed for 96 h to an environmentally relevant concentration (1 nM), similar for each drug, corresponding to the level measured in surface waters. This choice allowed to focus on the genotoxic impact towards biomarkers useful to assess the potential of drugs. The biomarker results pointed out that a low environmental concentration of TMP, IBU, DCF and PCM has a slight cyto-genotoxic potential on zebra mussel haemocytes. On opposite, 96 h exposure to 1 nM of TCS was able to significantly increase both primary and fixed genetic damage, as well as to induce significant (p<0.01) destabilization in lysosome membranes, suggesting an intense stress in treated bivalves. Even if our data highlighted that TCS appears to present a superior genotoxic potential of PPCPs used in this study, the next step of this research will be the analysis of the chemical ratio dependencies.
action and physical-chemical properties. Next to this, endocrine-disruptive activities are known to be observed for a few of the tested PPCPs. The obtained data indicate which of the measured GST activity. No changes in LDH activities were notice for embryos and adults exposed to AMX. Antibiotic seem not provoke alterations in liver xenobiotic metabolism at the dosage and time of exposure tested, as expected. Embryos seem to be much less responsive than adults to AMX and OTC short term exposure. Linkages between AMX and OTC exposure may thus influence the measured GST activity. No changes in LDH activities were notice for embryos and adults exposed to AMX. Antibiotic seem not provoke alterations in liver xenobiotic metabolism at the dosage and time of exposure tested, as expected. Embryos seem to be much less responsive than adults to AMX and OTC short term exposure. Linkages between AMX and OTC exposure may thus influence the measured GST activity.

As a conclusion, an exhaustive risk assessment is necessary to determine the maximum allowable concentrations in water of personal care products to preserve children's health.

TU 094
Comparative biomarkers responses of zebrafish life stages exposed to oxytetracycline and amoxicillin
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Oxytetracycline (OTC) and amoxicillin (AMX) are antibiotics that are extensively used worldwide. Both compounds are applied in fish and shrimp farming practices, and also used for human and veterinary medicine. Their systemic application has become a threat for both human and environmental health. Considering this scenario, our study aims at assessing sub lethal effects of AMX and OTC in embryos and adults of zebrafish. To achieve these objectives a battery of biomarkers were analysed in adults (liver, head, muscle, gill tissues) and embryos (whole embryo homogenates) exposed to these chemicals. Representative biomarkers include metabolic pathways genes (LDH), cytochrome p450 (CYP), hepatic catalase (CAT), lactate dehydrogenase (LDH), and glutathione S-transferase (GST). No differences in the mortality rate were obtained between embryos and adults for AMX and OTC treated concentrations. Adults exposed to OTC showed inhibition of the oxidative stress enzyme CAT in head tissues (96 h-LOECAThead = 25 mL/g) and an increase in the detoxification phase II enzyme GST (96 h-LOEGSTMuscle = 10 mL/g). OTC exposure also induced the GST activity in embryos at the highest concentrations (96 h-LOECSTembryos = 100 mL/g). LDH in adults showed a range of responses according to the different organs analysed: being inhibited in the head tissues (96 h-LOEHLHindhead = 50 mL/g) and increased in the muscle (96 h-LOEHLHMuscle = 10 mL/g) and liver (96 h-LOEHLHliver = 10 mL/g) as well as in embryos (96 h-LOECSTembryos = 150 mL/g). Adults exposed to AMX showed an inhibition of CAT in head and gills (96 h-LOECAThead = 1 mL/g and 96 h-LOEGLGills = 25 mL/g). An inhibition pattern was also found in GST head tissues (96 h-LOEGSThead = 1 mL/g). However, in the gills, muscle and embryos (96 h-LOEGSTMuscle = 1 mL/g, 96 h-LOEGSTgills = 10 mL/g and 96 h-LOECSTembryos = 380 mL/g) an induction pattern was observed in the measured GST activity. No changes in LDH activities were observed in embryos and adults exposed to AMX. Antibiotic seem not provoke alterations in liver xenobiotic metabolism. Each dose is connected to an automated, computer controlled data capture system, which records the oxygen consumption by samples of activated sludge in each incubation chamber after aeration has been terminated and the headspace purged with nitrogen. We describe in detail our operation with the use of this sensitive equipment and the results that we have obtained with the reference substance 3-5 dichlorophenol and other xenobiotics as test substances. The obtained results were used to derive an exposure guideline for activated sludge respiration in OECD Method 209.

TU 095
Gemfibrozil and naproxen biodegradation in a river water ecosystem
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Incomplete removal during wastewater biological treatments is the main source of surface water contamination by pharmaceuticals. Degradation of a chemical in the aquatic environment has been shown to be limited by the chemical properties of the molecule. However, the extent of biodegradation depends on many factors such as temperature, 

TU 090 Effects of chronic exposure to pharmaceuticals on aquatic biota: an experimental study
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Pharmaceuticals are widely used compounds and many of them will eventually end up in the aquatic environment where they might affect various organisms. Two commonly used antibiotics that have been shown to be active in the aquatic environment are Sulfamethoxazole and Ciprofloxacin. As they are used to efficiently treat bacterial infections there is a great risk that they affect environmental microorganisms similar to their target organisms if they end up in the environment. Therefore, the concentration-response relationship of these two substances was investigated on natural marine biofilm communities (periphyton) mainly composed by algae and bacteria. Gliss discs were submerged in the sea between the small harbor of Fiskebackskil on the Swedish west coast and the biotous colonized the substrate during a week. Thereafter the biotous were exposed to the two antibiotics (5mmol/L - 9mmol/L) in the lab using the semi static SWIFT periphyton test during 72 hours. Antibiotic-induced effects on the compounds were analyzed for both bacteria and algae. Pigments were extracted from the algic part of the communities and the composition was analyzed using high performance liquid chromatography. The pigment composition was used as a way to estimate structural changes in the community. Effects on the periphyton bacteria were measured using the so called Biolog Ecoplates® methodology. The communities ability to utilize different carbon sources is used as a measure on community function.
ecosystem depends on a variety of factors, including compound properties and environmental factors and above all the presence of a natural microbial community able to degrade it via metabolic and/or co-metabolic pathways. Although pharmaceutical and therapeutic products are widely found in the natural environment, the ecological effects on receiving ecosystems remain largely unknown.

Ibuprofen, a nonsteroidal anti-inflammatory drug and gemfibrozil, a fibrate drug used as lipid regulator, have been found in several natural waters. They were also found in inflow from a municipal wastewater treatment plant inside the city of Rome in and in the receiving River Tiber. For this reason we studied the degradation of these pharmaceuticals in microcosm studies using natural river water. The biotic and abiotic degradation (in terms of the disappearance of the 50% of the initial concentration) of naproxen and gemfibrozil were evaluated in microbiologically active natural non-human in surface waters. The objectives of this study were to determine caffeine presence in the Danube River samples and to evaluate its stability during two months storage of samples on 4°C. Analysis was performed by solid-phase extraction (SPE) followed by new developed reversed phase high performance liquid chromatography (RP-HPLC) method. The chromatography was using a Zorbax Eclipse XDB-C8 column (150 mm × 4.6 mm, i.d., 5 μm particle size) at 25°C, with a mobile phase of 0.1% TFA in water (pH 8) - acetonitrile (85:15, v/v). The flow rate was 1 mL/min, and detection by DAD at 273 nm. Samples were collected in July 2011 on seven different locations of the Danube River on a territory of Novi Sad, Serbia. Caffeine amount ranged between 0.84 ng/L on the 1st day and 0.52 ng/L on the 30th day after sample collection. On the 60th day after sampling caffeine was under the limit of detection in all water samples.

Presence of caffeine confirmed the existence of human wastewater in the Danube River. Highest values were obtained in sample collected from location near the water supply source “Ratno ostrov”, Novi Sad. Obtained results due to caffeine amounts significantly decreased during two months storage of samples on 4°C most likely as its degradation.

The work was supported by Ministry of Education and Sciences, Republic of Serbia (II46009) and NATO Science for Peace Project ‘Drinking Water Quality Risk Assessment and Prevention in Novi Sad municipality, Serbia’ (SEPESAPF 984087).

TU 097
The effects of ibuprofen exposure on fathead minnows (Pimephales promelas).

We have identified the COX-1, COX-2a and COX-2b genes in the fathead minnow (Pimephales promelas). To assess the uptake and effect of ibuprofen exposure, fathead minnows were exposed to ibuprofen concentrations close to nominal (105 ± 2.6 and 502 ± 49 μgL⁻¹) and 360-fold (500 μgL⁻¹) higher than the non-steroidal anti-inflammation drug, ibuprofen, has been reported in aquatic waters in the UK at concentrations ranging between 3-27 μgL⁻¹. Ibuprofen is a widely prescribed use and over-the-counter medication, treating pain, inflammation and fever by reducing the level of prostaglandins through non-selective inhibition of the enzyme cyclooxygenase (COX). COX exists in two isoforms; the constitutively expressed COX-1 and the inducible COX-2. In order to evaluate whether pharmaceuticals pose a threat to the aquatic environment, we are testing the hypothesis that any potential effects will be related to the Mode-of-Action of the drug and will be seen at plasma levels in non-target organisms similar to human therapeutic levels.

We have identified the COX-1, COX-2a and COX-2b genes in the fathead minnow (Pimephales promelas). To assess the uptake and effect of ibuprofen exposure, fathead minnows were exposed to ibuprofen in water at 1, 2, 4, 8 and 16 hours after exposure. Gills, gonads, gut, liver, muscle and brain were collected to determine the effects (if any) on COX gene expression. RNA isolated from tissues was reverse transcribed to cDNA and amplified by qPCR using specific primers to the COX genes and reference genes (18S and β-actin). Initial results indicate changes in COX gene expression following ibuprofen exposure.

Water samples were also collected to determine ibuprofen concentrations in the fish and the exposure water, using mass spectrometry. This confirmed that fish were exposed to ibuprofen concentrations close to nominal (105 ± 2.6 and 302 ± 49 μgL⁻¹, respectively). Analysis of the plasma revealed uptake of ibuprofen to be up to 9-fold higher than the nominal concentration. Vancomycin was used as a control for direct release of ibuprofen from the microsomal fraction. Plasma and water samples were also collected to determine ibuprofen concentrations in the fish and the exposure water, using mass spectrometry. This confirmed that fish were exposed to ibuprofen concentrations close to nominal (105 ± 2.6 and 302 ± 49 μgL⁻¹, respectively). Analysis of the plasma revealed uptake of ibuprofen to be up to 9-fold higher than the nominal concentration. Vancomycin was used as a control for direct release of ibuprofen from the microsomal fraction.

Large-scale spatial-temporal modeling of historical pesticides applications.

In the European Regulatory context of inclusion of active substances in the positive list of Regulation (EC) N° 1107/2009, authorizing their use as pesticides, studies conducted in US are usually not recommended because of significant differences in experimental design, soil specificities and objectives when compared to OECD guidelines. However when a US soil metabolism study (conducted according to US-EPA guideline i.e. OPPTS 835.4100 using US soils) was conducted and submitted as part of the EU dossier, Regulatory authorities may ask for clarifications on soil metabolites, even formed under the specific conditions of this study type.

EM02P2 - Fate and exposure modelling
The objective of this work is to present a case study where apparent conflicting results were obtained between US-type soil metabolism and EU-type soil metabolism studies: In the US study, additionally to one major metabolite M, two metabolites (m1 and m2) were considered to be formed directly from the parent in the metabolic pathway proposed by the laboratory in amounts continuously increasing up to around 7% of the applied radioactivity until the end of the one year incubation. These results were conservative for the EU studies where only M was identified. Kinetics optimization with ModelMaker software and statistical analysis of the data according to the recommendations of the FOCUS Kinetics Workgroup (2006) were performed. The kinetic evaluation demonstrated that, using a new metabolic pathway, this optimization of kinetic data leads to an acceptable error. Therefore, these soil metabolites were found not to be environmentally relevant according to EU guidance. This justifies the need to perform additional kinetic investigation of the metabolism of pesticides so as to set up properly the degradation pathway.

TU 102

Determination of plant uptake factors for pesticide fate modelling

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The data on PUF show that the use of the FOCUS default of 0.5 is a very conservative approach in terms of leaching assessment. For the test compounds exhibiting ionic to lipophilic properties of varying degree, PUFs were derived for four substances in each of three different crops (Tomato, Wheat, Maize).

In the kinetic experimental set-up intact plants were exposed between 8 and 11 days to a nutrient solution (pH 6) spiked with the radiolabelled test compound. Volume uptake and concentration in the nutrient solution were measured regularly over the experimental period. By sealing the test vessel it was assured that the only loss process was evaporation from the system for water and chemical was plant uptake.

As a result of the study, the PUF was always well above the FOCUS default value of 0.5 (FOCUS, 2009). Generally, a small variation between plant species was observed. The experimental results could be verified with a simple plant uptake model. The data on PUF show that the use of the FOCUS default of 0.5 is a very conservative approach in terms of leaching assessment. For the test compounds exhibiting ionic to lipophilic properties of varying degree, PUFs were derived for four substances in each of three different crops (Tomato, Wheat, Maize).

TU 103

Development of a French national tool for pesticide risk assessment in the context of the water framework directive

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The main objective of the Water Framework Directive (2000/60/EC) is to achieve good ecological status in all surface and groundwater bodies in the EU by 2015. However, it is already clear that this goal cannot be achieved by 2015 for a number of reasons.

Pesticide risk evaluation for surface waters in the EU is based on the FOCUS standard scenarios ditch, stream, and pond, which are characterized by fixed amounts of water for dilution of pesticide input. The representativity of this water bodies for existing waters in real landscapes of the EU was never verified, and there is some doubt that the FOCUS standard scenarios reflect the "realistic worst case" appropriate. Given the fact that the vast majority of water bodies are flowing waters and not stagnant ditches, a more realistic pesticide exposure assessment has to respect some basic properties of rivers:

(i) In flowing waters, the hydrodynamic dispersion lowers the peak concentration but spreads the long-tudinal extension of an initial substance pulse.
(ii) Pesticide applications on fields along a river stretch should be located such as a water package moving downstream the water course may be affected by depositions via spray drift, runoff and tile drainage, respectively, more than one time.
(iii) The locations of pesticide input and the sites where ecotoxicological effects may occur are apart from each other.

The poster presents results of the PECFOCUS standard scenarios ditch, stream, and pond, which are characterized by fixed amounts of water for dilution of pesticide input. The representativity of this water bodies for existing waters in real landscapes of the EU was never verified, and there is some doubt that the FOCUS standard scenarios reflect the "realistic worst case" appropriate. Given the fact that the vast majority of water bodies are flowing waters and not stagnant ditches, a more realistic pesticide exposure assessment has to respect some basic properties of rivers:

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The general procedures for calculating degradation rates are well known but the assumptions made during this process can appreciably affect the results. Normalisation of field degradation data may need to be performed to obtain reliable degradation parameters and to be used for modelling purposes. In the field, the persistence of pesticides is affected by the varying environment, i.e. soil moisture and temperature having a significant effect on the bioactivity of the criterion needs to be chosen with care. Studies on the speed of movement of pesticides in the soil, depending on substance and soil specific properties and rainfall, may help to improve the existing models. A validation of the national tiered approach requires a prediction of endpoints from modelling and measured leachate concentrations from outdoor lysimeter studies (tier 3). Further investigation is planned on the critical points, how the short duration of lysimeter studies in combination with a single application finally affects the prediction of the impact of pesticides under realistic conditions. However, the implementation of the new FOCUS PELMO model in the German authorisation procedure in combination with the adapted selection of modelling endpoints requires a validation of the national tiered approach. A current research project, predicted and measured leachate concentrations from modelling and outdoor lysimeter studies, may help to improve the existing models. Further investigation is planned on the critical points, how the short duration of lysimeter studies in combination with a single application finally affects the prediction of the impact of pesticides under realistic conditions. However, the implementation of the new FOCUS PELMO model in the German authorisation procedure in combination with the adapted selection of modelling endpoints requires a validation of the national tiered approach. A current research project, predicted and measured leachate concentrations from modelling and outdoor lysimeter studies, may help to improve the existing models.

In the past years, the time-step normalisation procedure as described by FOCUS (2006) has become popular in the EU registration. This procedure assumes that the decline in the field can be described well by numerical models that assume first-order degradation kinetics. The procedure implies that the decline curve after normalisation can be used directly to estimate the DegT50 matrix of the top soil at 20°C and pF = 2.6. This matrix, however, needs to be combined with a leaching model (here: FOCUSPELMO 4) to calculate DT50 values that could describe the outdoor leaching of a compound under realistic conditions. In the registration procedure in Germany, the risk assessment for the leaching potential of plant protection products into groundwater is based on modelling results using simulated leaching concentrations of the FOCUS ground water scenario from the latest version of the FOCUS RRCM model (tier 2). Dependencies of pesticide sorption and degradation data from different soil properties as well as parameter variability are considered in the end point selection for modelling, which is in line with the FOCUS recommendations. The measured leachate concentrations for active substances and/or metabolites from outdoor lysimeter studies are accepted as higher tier endpoints in the risk assessment and override predicted concentrations from simulation runs (tier 3). This is generally justified by comparable soil and climate conditions in the Hamburch scenario and the experimental data, but the assumption that measured leachate concentrations can be directly used to estimate DT50 is not supported. Inverse modelling simulations are used to vary the input parameters Koc and DT50 until the leaching model shows the same results as the experiment. Generally, 2 steps have to be conducted when performing inverse modelling simulations. Firstly, the hydrology in soil is optimised, followed by the optimisation of pesticide fate. The software InversePELMO is able to provide both programs as a built-in module and the simulation model with the necessary input files in the correct format. InversePELMO has also a built-in module to perform standard statistical tests to check the quality of the optimisation such as the determination of the ‘FOCUS error’ at which the chi2 error decreases as described in FOCUS (2006). It was also tested that PEST works under all relevant windows systems (XP, VISTA and 7) in a 32 bit as well as 64 bit version. The results of InversePELMO can be used to make predictions of likely behaviour if the study had been conducted over a longer time period, translations of the lysimeter results to a different situation with respect to the environmental conditions (e.g. different climate), translations of the lysimeter result to a different situation with respect to the application pattern of the substance (e.g. change of the rate), and the use of optimised parameter settings for a refined reduced tier 1 simulation.
full-field environmental programme is barely affordable for a new active ingredient let alone for a single formulation. Simulation models are the primary basis for regulating pesticides against ground- and surface-water protection standards, but the current models cannot explicitly represent plants or their roots, or the effects of formulations and formulation placement strategies (e.g. slow-release microcapsules and seed treatments). Other models exist that represent individual components of the system in a more satisfactory way.

Current models used for pesticide registration within the EU are 1-dimensional and have a simplified representation of root development and uptake of pesticides. Conversely, plant growth and uptake models can have sophisticated representation of plant processes, but often do not consider pesticide dynamics. An ongoing project has created a conceptual framework for representing local pesticide concentrations in the whole soil-plant system, and will next produce a spatially-explicit simulation model. By relating local concentrations of pesticides to product efficacy, it will be possible to use the model to design better active ingredients, formulations and placement strategies. The new model will also enable the environmental benefit of advanced formulations and placement strategies to be quantified in a cost-effective way for the first time. This presentation will introduce the project to the scientific community, overview results from the first year of work, and discuss anticipated outcomes and the potential benefits to the scientific and regulatory communities.

The project co-funded by the Technology Strategy Board, the Department of Environment, Food and Rural Affairs (DEFRA), and the Biotechnology and Biological Sciences Research Council (BBSRC).

TU 112

Last advances and perspective for a better risk assessment of the tropical use of Protection Plant Products in France

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Risk assessment of water contamination required prior the pesticides registration for French overseas territories is currently conducted using tools and guidance documents established for EU context although the agro-pedoclimatic conditions and transport of pesticide in these territories underlines that (1) a minimum of four scenarios (2 soils: arable and nitrous and 2 crops: banana and sugar cane) would be necessary for the risk assessment of the main uses of the French overseas territories and (2) the models to be used for predicting transport of pesticide have to account for preferential flow and specific redistribution of rainfall by crops canopy for banana. For water surface contamination, the module for surface water fate would likely to be initially simplified to a simple static receiving water body of dimensions defined consistently with French overseas territories and protection goals. Finally, the main features of two models - currently under test- representing surface and subsurface flow of water and pesticides in banana crops on tropical volcanic soils are summarized and the schedule of a 2 year research project (2012-2014) to adapt them for regulatory purposes introduced.

TU 113

Simulation of the herbicides fluafenacet and metazachlor in a lowland catchment with the model SWAT - A sensitivity analysis

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Spatial differentiation is a topic of increasing interest within ecological risk assessment (ERA) and Life Cycle Impact Assessment (LCIA). A key issue to be addressed in the impact assessment methods is to determine impact factors as a consequence of spatial detail and uncertainties related to the use of generic impact factors when the exact location of the activities is unknown. For freshwater ecotoxicity evaluations were performed in order to assess the relative influence of scale in calculation of LCIA characterization factors, comparing them with the result of site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the site specific risk assessment as a consequence of the site specific risk assessment.
1. Introduction
This poster presents intake factors (IF) and characterization factors (CF) regarding human health effects for the pollutants NMVOC, NOx and SO2 accounting for health impacts due to ozone. Research on life cycle impact assessment (LCIA) for transboundary pollutants have been reviewed and it is suggested that spatially differentiated fate and effects models should be created. The present work aims to fill the gap of consistent global modelling by developing an approach to derive globally applicable and spatially explicit values, and by suggesting the spatially explicit, LCIA methods and characterization factors.

2. Materials and Methods
Within the ongoing EU-funded project LC-IMPACT (2010-2012) the “Development and application of environmental Life Cycle Impact Assessment Methods for imProved sustAnibility characterisation of technologies” is pursued. Within the area of “Non-toxic pollutant impacts” the objective is to develop globally applicable, but spatially explicit, LCIA methods and characterization factors.

Within the TM5-FASST modelling framework, the world is divided into 36 regions. Each region serves as a source region and each grid cell (resolution =1°×1°) of the whole world serves as receptor region. Population data and concentration response functions are applied in order to calculate the IF and relevant diseases. Finally, the health impacts are weighted in order to get DALYS per unit of emission, and different external cost estimates, by including and excluding equity weighting.

3. Results
A detailed comparison will be made between if's derived by the TM5-FASST model and by the EcoSenseWeb integrated assessment tool in order to assess the inherent uncertainties and implicit variability in the estimates. IFs and CFs are calculated for several countries and larger regions. Finally, IFs and CFs suggested in different LCIA methods have been compared with the findings of this work

Acknowledgements
The author thanks the colleagues of the LC-IMPACT and the European Union for funding. The author thanks the JRC-ISPRA, Institute for Environment and Sustainability (IES), Climate Change Unit (CCU) for kindly making the global scale assessment based on the TM5-FASST tool possible.

TU 119
Environmental impacts of thermal emission to freshwater: Spatially explicit fate and effect modelling
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Power production is a crucial sector causing heavy impact on freshwater bodies by releasing enormous amounts of heat in once-through cooling systems. So far only few approaches have addressed impact on aquatic environment related to this issue and there is no study providing fate and effect model for assessing the impacts on a spatial explicit level and broad coverage. We started our research based on a detailed study assessing impacts of thermal emissions from different sizes of power plants in Switzerland and extracted the most sensitive parameters to derive a generic model of combined fate and effect model for the US. We created a 0.5 arc degree grid accounting for water temperatures, river flow, river width, flow velocity and distance to sea in order to model fate and effect of thermal emissions from different sizes of power plants. Various data were collected from different essential parts and different impact models are applied to cover points without measured data, allowing accounting for uncertainties. We further investigated uncertainties of the characterization factors by varying input parameters and the model assumptions, including the assessment of the affected species. The thermal impacts show significant spatial variability depending on the water body where the cooling water is discharged and the amount of heat released, because the impacts are non-linear. Furthermore this project illustrates how fate and effect model can be integrated to improve the assessment of spatially differentiated impact assessment considerably.

TU 120
Including the influence of worldwide crop cultivation on ecosystem services in life cycle assessment
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Land use is increasing worldwide leading to changes in the ecosystem. Changes in ecosystem services allows for quantification of the impact that each land use activity causes to the environment. This can be achieved through the quantification of changes in ecosystem services. Ecosystem services include carbon sequestration, nutrient cycling, erosion regulation and biotic production potential. The goal of this research is to develop indicators that estimate changes in ecosystem services due to changes in selected land use activities namely, cultivation of rapeseed, soybean and sugarcane, on a global scale. In Life Cycle Impact Assessment (LCIA) these indicators are known as characterization factors (CFs). To this research we focus on crop cultivation and the present work are focusing on the growth processes of crop, water and nutrient cycles. The cultivation of the crops is simulated using the Environmental Policy Integrated Climate model (EPIC). EPIC is a spatial and temporal agri-environmental model which simulates the biophysical impacts on homogenous response units characterized by altitude, slope and soil class. Inputs for EPIC simulations include weather and soil profile data, and information on land use, land cover, and crop management. By simulating processes of crop growth, water and nutrient cycles, EPIC produces, among its outputs, estimates of crop yield, sediment transportation and soil carbon sequestration. The characterization factors will be aggregated to country or biome level. Comparisons will be made between results from different spatial aggregation levels, and different management systems regarding input of fertilizer or irrigation.

RAO2P - Approaches for comparative hazard and risk assessment of chemicals

TU 121
Health risk assessment of chlorpyrifos with rice farmers in Vietnam
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Vietnam is an agricultural developing country with over 80 per cent of workers employed in agriculture, mainly rice farming cultivation. Farmers use back-pack reservoirs with hand pumps to apply pesticides, but their knowledge of safety in pesticide application is low. Chlorpyrifos is the most common organophosphate insecticide registered for agricultural use in Vietnam, but health risk assessment of chlorpyrifos use has not been carried out and limited investigation in Vietnam and other developing countries. The objective of this study is to evaluate the health risk of chlorpyrifos exposure to a typical group of rice farmers after application in Vietnam, using a probabilistic approach. Biological monitoring of rice farmers was used to estimate chlorpyrifos exposure from all pathways during application. Urine samples (24 h) were collected from farmers (18) one day prior to application and over 5 days post-application, and then analysed for TCP, the main metabolite of chlorpyrifos, using HPLC-MS/MS. Urinary TCP levels were converted into an absorbed daily dose (ADD) of chlorpyrifos for each farmer. The health risk of chlorpyrifos exposure for the rice farmers was characterised by comparing exposure doses (ADD) with acute guideline doses for chlorpyrifos used by USA and Australian agencies.

Post-application chlorpyrifos ADD of farmers varied from 0.4-9.4 µg/kg/d, with a 50th percentile exposure level of 8 µg/kg/d which was about 80-fold higher than the 50th levels were converted into an absorbed daily dose (ADD) of chlorpyrifos for each farmer. The health risk of chlorpyrifos exposure for the rice farmers was characterised by comparing exposure doses (ADD) with acute guideline doses for chlorpyrifos used by USA and Australian agencies.

This case study in Vietnam has shown that rice farmers are at a high risk of chlorpyrifos exposure and resultant adverse health effects, mostly neurotoxicity.

TU 122
A soil ingestion pilot study of a population following a traditional lifestyle typical of rural or wilderness areas
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The relatively few soil ingestion studies underpinning the recommended soil ingestion rates for contaminated site human health risk assessments (HHRAs) that have been conducted to date assessed soil ingestion in children living in urban or suburban areas of the United States, and to a lesser extent, Europe. However, the lifestyle of populations living in North American urban and suburban environments is expected to involve limited direct contact with soil. Conversely, many populations, such as indigenous and Aboriginal peoples residing in rural and wilderness areas of North America and worldwide, practice traditional land use, and participate in activities that increase the frequency of direct contact with soil. Qualitative soil exposure assessments to estimate ingestion rates for Aboriginal populations inhabiting wilderness areas suggest that high levels of soil ingestion (i.e., 400 mg d⁻¹ quantities) may occur, and these levels are many times greater than those recommended by regulatory agencies for use in HHRAs. A recent assessment of subsistence selected from a wilderness community in Canada was conducted using mass balance tracer methods to estimate ingestion and the results compared with previous soil ingestion studies and regulatory guidelines for the soil ingestion rates used in HHRAs of contaminated sites. A pilot study of 7 subjects belonging to the Xeni Gwet’in First Nation community living in the Nemiah Valley of British Columbia was conducted over a 3-week period. The study provided the first quantitative soil ingestion estimates of an Aboriginal population following a traditional lifestyle. The soil ingestion rates estimated in this study were much higher than those currently recommended for HHRAs of adults, and higher than those obtained in most previous studies of adults. However, the soil ingestion rates measured were much lower than earlier qualitative assessments of subsistence lifestyles.

TU 123
Health risk assessment and economic damage due to environmental pollution in a large industrial centre
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A health assessment in Perm, an industrial city in European Russia, has been performed using the WHO methodology. The findings indicate spatially differentiated chronic exposure in 95% of the Perm residents to various chemicals (formaldehyde, ethyl benzene, benzene, nitrogen dioxide, etc.) at concentrations exceeding maximum permissible concentrations (MPC) (acute exposure) and maximum permissible average daily concentrations, by 16.7 and 13.5 fold, respectively. More than 65% of the exposed Perm residents live in houses which do not meet the standards, i.e. trichloromethane, residual chlorine, iron, manganese and other impurities levels exceed MPC by 2.3 fold. We have observed an increase in acute and chronic hazard indices up to 30 fold for 14 out of the 17 analysed critical body systems and organs. Almost all of the city population is at an unacceptable environmental risk for respiratory, eyesight, hematological and immune disorders as well as central nervous system impairments. More than 37% of the Perm population (11.97% for women and 30.82% for men) live in environments exceeding the MPCs. The main risk factor is inhalation exposure. The presence of the above mentioned risks has been proven by the identification of contaminants in the blood of the exposed subjects and the deterioration of laboratory, clinical and functional health parameters. We have revealed a true relationship between elevated blood levels of acetaldehyde, formaldehyde, manganese, nickel, chromium, etc. and blood, immune, hematological and other health indicators. We have determined reference levels of the chemical compounds in blood, i.e. acetaldehyde -0.286 mg/dL, manganese -0.09 mg/dL, chromium -0.021 mg/dL, and formaldehyde -0.286 mg/dL. We have identified NOAEL values in the air: Ni - 0.00009 mg/m³, Cr - 0.00015 mg/m³, Mn - 0.00025 mg/m³, Acetaldehyde -0.002 mg/m³, Benzene - 0.003 mg/m³; Chloroform reference dose in drinking water: 0.0004 mg/L. We suggest that priority environmental and acute risk criteria cause 1,246 new additional cases of respiratory diseases. The total economic damage for respiratory diseases is € 1.3 million per year. Acceptable risk levels can be reached by reducing industrial emissions of 19 industrial companies, using traffic optimisation and clean fuel and improving the water supply system quality and water treatment.

TU 124
Snail watch: a tool to assess the risk of metal transfer taking into account their bioavailability

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Current evaluation of soil quality, generally based on physico-chemical characteristics, does not integrate the biological aspect and so the risk of metal transfer to organisms. The implementation of a soil chemical quality assessment allows evaluating the bioavailability of metals and then their risk of transfer.

The aim of this study is firstly to determine the internal concentrations of reference (CIRef) of metallic contaminants (Cd, Pb, As, Cr, Cu and Zn) in Cantareus aspersus snails. Then, CIRef are used to identify abnormal metal transfer to snails. Secondly, to estimate the soil characteristics influence on metal accumulation using multivariate equations. Our objective is to evaluate Risk of Transfer of MEtals (ERTIME) to snails to determine management priorities that take into account physico-chemical and biological parameters of metal availability.

CIRef have been established on the basis of concentrations in the snails exposed on unpolluted sites for 28 days i.e. snail watch. In snails exposed on other sites, when the value of CIRef is higher than the observed above the CIRef, the site will be considered as potential sites of metal transfer. Most of the time, the use of these equations has enabled to determine pH, organic carbon content and iron oxides as parameters influencing metal bioavailability.


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Environmental Quality Standards (EQSs) are a key tool under the Water Framework Directive (WFD) to help assess the chemical status of water bodies. However it is important that EQSs are developed for substances that pose the greatest risk to the aquatic environment, and that emerging contaminants are adequately considered.

Various methods for prioritising chemicals have been described, incorporating both exposure and effects. Approaches that prioritise on the basis of risk often require significant inputs, even a draft EQS, or detailed exposure modelling. We describe a simple approach to prioritisation that requires only modest inputs and assigns chemicals to different risk categories.

Classification based on exposure and hazard are then combined and chemicals assigned to risk categories. Those assigned to the highest risk category are taken forward as potential candidates for EQS development.

Almost a third of the chemicals considered could not be assigned a risk ranking due to a lack of data. This is of particular concern for emerging contaminants where both hazard and exposure data are often limited. Lack of exposure or hazard data contributed equally to the assignment of ‘Insufficient information’. Predictive tools such as QSARs or ‘read across’ techniques may have a useful role in filling some gaps in hazard data. Gaps in exposure data may be filled by gathering monitoring data; in this respect the scheme has helped identify monitoring needs that may lead to identifying future EQS candidates.

The prioritisation methodology is not suitable for metals and other inorganic chemicals because of biases in the way that fate and behaviour are dealt with e.g. measures of the tool has helped identify monitoring needs that may lead to identifying future EQS candidates.

The need to use biological tools that take into account physico-chemical and biological parameters of metal bioavailability.

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The need to use biological tools that take into account physico-chemical and biological parameters of metal bioavailability.
Efficient toxicity testing when test information is uncertain: a stochastic cost-effectiveness analysis

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Changing policy settings for chemicals' risk management, for example in Europe and in the United States, have led to increasing awareness of the urgent need for more efficient testing of chemicals. The economic concept of efficiency calls for allocating scarce resources (e.g., money, time, human skills) such that output is maximised. Hence, if a toxicological endpoint different testing options exist, a decision maker must identify the testing strategy that provides the highest information gain per unit of cost. This clearly, requires to weigh the costs associated with a testing strategy, usually measured in monetary terms, against the information gains from testing. The latter are commonly expressed in terms of the a test's predictive performance. Cost-effectiveness analysis (CEA) provides a powerful tool for solving this problem. CEA allows for a comparative evaluation of different testing trade-offs between costs and information gains for different testing methods and strategies. While CEA has been widely used in medical domains, for example in medical decision-making, there are only few attempts to apply CEA to testing in toxicology. These studies share the assumption that information gains from testing, measured in terms of a test's predictive performance, are known. The predictive performance of testing methods is, however, uncertain, irrespective of what type of testing method (e.g. a "gold standard" in vivo test, an in vitro test, or a "non-testing" method) is used. Our study, therefore, moves beyond existing deterministic CEA modelled in toxicology by developing a stochastic approach to CEA that accounts for the uncertainty about the quality of test information. The aim is to allow for a more robust and transparent evaluation of testing methods and strategies both within and across endpoints. This guides decision-makers in coherently selecting the testing strategy or provider that provides the highest information gains per unit of cost. Another contribution of the study is to offer a critical synopsis of data and research needs in order to apply our approach to various endpoints and to make it operational in the regulatory context of REACH.
An environmental specimen bank (ESB) is an archive for biological samples that can be used to perform (traditionally chemical) analyses to obtain information about long-term variability and temporal trends in the chemistry of the biota and the ecosystem quality. Aware that chemical endpoints alone are not enough for ecosystem health analysis, the Biscay Bay Environmental Biospecimen Bank (BBEBB) was designed including specimens for biological endpoints, say bioplyes for histopathology/immunochemistry, cryo-specimens for molecular/cellular analysis, and dried mineralized tissues (shells/lololiths) for biometric determinations. Banking methods and specimen sampling and processing are being adapted for these needs. In a first practical application, a retrospective study was performed using the ‘ Mussel Shell Section’ of the BBE88 (2001-2010) localities covering the area of a geographical distribution of Mytilus galloprovincialis in the North Berin coast. Changes in shell size (length, width, height), allometry and shell weight (SW) were analyzed, and the shell thickness was estimated after regression of the log SW against SSF (Shell Size Factor, LxWxH in mm). Overall, shell growth exhibited a decreasing trend during the study period with a seemingly more marked arrest growth in 2003-2006. These results are discussed in terms of interactions between the global environment trends and the local consequences of the Prestige oil spill. However, since only specimens of L=3.5-4.5 cm were analyzed in most cases and mussels of the same shell length can belong to different age classes depending on the geographical variability and the influence of environmental conditions for a given locality, the incidence of the age in the interpretation of the obtained results is also discussed. In summary, the development of Mussel Shell Sections in environmental biospecimen banks is highly recommended as they may offer an excellent opportunity for retrospective monitoring after low cost processing and storage. In addition, our results also suggest that mussel watch monitoring programs and ESB archives should be adapted on the basis of samples of same-age rather same-length mussels. This study was supported by the Government of the Basque Country through K-EGOKITZEN project.

TU 135 Using banked seabird eggs for determining geographic patterns of trace elements in lake regions R.S. Pugh1, W.C. Davis2, D.G. Kottwitz, P. Hsu
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Seawater from the ocean could influence the concentration of metals in the area, or it could be similar to that if other areas. Selenium and arsenic occur naturally in the environment but they are also known to be toxic in excess of trace levels. During the 1990s, elevated concentrations of arsenic were reported in liver tissue of ringed seals taken by Alaska Native hunters in Norton Sound, Alaska. To determine whether eggs of colonial seabirds might be good monitoring matrices for both arsenic and selenium in marine environments, a total of 17 colonies of 10 different species were sampled in 2008 and banked at the US Marine Environmental Specimen Bank (Marine ESB). These eggs were collected from several colonies located within Norton Sound as well as long-term monitoring sites in two other major Alaska marine regions, St. Lazaria Island in the Gulf of Alaska and St. George Island in the Bering Sea and were from three species: common murre (Uria aalge), thick-billed murre (U. lomvia) and glaucous gulls (Larus hyperboreus). Egg samples were analyzed using collision cell kinetic energy discrimination inductively coupled plasma mass spectrometry (ICP-MS). Total arsenic mass fractions ranged from 0.015 µg/kg to 0.320 µg/kg while the levels of Se were higher from 0.411 µg/kg to 1.017 µg/kg. As and Se levels were higher in most of the samples collected from the Norton Sound colonies compared to other colonies. Norton Sound is located in a highly mineralized region of Alaska and is an area of historical gold-mining that continues today and could be a contributing factor to the bioaccumulation of arsenic and selenium in this region. In addition, As mass fractions were also measured in 43 common murre and glaucous gull eggs collected in 1999, 2000, and 2005 from colonies located throughout the Bering Sea, including 4 of the same species as those sampled in 2008. The As mass fractions ranged from 0.065 µg/kg to 0.349 µg/kg and were also more elevated in the Norton Sound colonies than other colonies in the Bering Sea. The data reported here illustrates that seabird eggs can be a useful monitoring tool to help determine geographic patterns of trace elements in marine regions, especially the sub-Arctic regions of Alaska.

TU 136 Specimen security in long-term species banking - zebra mussel example in the German ESB M. Paulus1, D. Teubner2, T. Bartel-Steinbach1, T. Weimann1, T. Bartel-Steinbach1, K. Tarricone1, G. Wagner1, A. Körner1, M. Veith1
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In the past few years many areas have seen a dramatic drop in zebra mussel numbers. Now, out of nowhere, what was once a successful model for monitoring waters is endangered of not producing a sufficient number of specimens for large samples. A possible cause is the receding eutrophication level of the waters and the resulting lack of food. Another invasive species, the quagga mussel (Dreissena bugensis), which has a stronger presence in many areas, is increasing the pressure on the zebra mussel populations too. Recent research has also shown that fertile hybrids between D. polymorpha and D. bugensis are possible in the wild - making biomonitoring even more difficult. Investigations into the current significance of the zebra mussel in long-term monitoring projects and environmental specimen banking programmes in the German ESB show that the zebra mussel provides valuable information but that accumulation in both species is also different, which is why D. bugensis and hybrids of both species have to be excluded from the results of monitoring studies using D. polymorpha for them to be comparable. Therefore, the most important task concerning D. polymorpha as a biomonitoring tool is to:
- find new species of other invasive species, which provide information about the zebra mussel ecological impact,
- develop a method to differentiate unequivocally between both species for large samples.

TU 137 Organic compounds in suspended particulate matter - results from the German environmental specimen bank K. Weinertmann1, C. Schröter-Kemm1, M. Ricken2
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Since 2003 increased particulate matter (PM) in the river systems of Rhine and Elbe has been sampled, processed and archived in the frame of the German Environmental Specimen Bank (ESB). Sampling is carried out with sedimentation boxes which are emptied monthly. At the end of the year monthly samples are lyophilized and pooled to a homogenate. From every homogenate about 200 subsamples are produced and stored in the ESB. The whole processing is conducted under deep frozen conditions. Some sub-samples are chemical characterized for inorganic substances, CHCs and PAHs. The results of the five years period 2003 - 2008 show considerable differences between the two river systems. The concentrations of PAH16 as well as of PCB6 and DDX were more elevated in the Norton Sound colonies than other colonies in the Bering Sea. The data reported here illustrates that seabird eggs can be a useful monitoring tool to help determine geographic patterns of trace elements in marine regions, especially the sub-Arctic regions of Alaska.

TU 138 Standardized freshwater mussel watch for monitoring of aquatic environments: implications and possibilities T. Yang1, J. Angelstorf1, M. Kottwitz, P. Hsu
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Mussel Watch is one of the most effective approaches to study the dynamics of aquatic pollution. Unfortunately, it utilization is usually limited to the depending on collection methods and the availability of specimens for large samples. A possible cause is the receding eutrophication level of the waters and the resulting lack of food. Another invasive species, the quagga mussel (Dreissena bugensis), which has a stronger presence in many areas, is increasing the pressure on the zebra mussel populations too. Recent research has also shown that fertile hybrids between D. polymorpha and D. bugensis are possible in the wild - making biomonitoring even more difficult. Investigations into the current significance of the zebra mussel in long-term monitoring projects and environmental specimen banking programmes in the German ESB show that the zebra mussel provides valuable information but that accumulation in both species is also different, which is why D. bugensis and hybrids of both species have to be excluded from the results of monitoring studies using D. polymorpha for them to be comparable. Therefore, the most important task concerning D. polymorpha as a biomonitoring tool is to:
- find new species of other invasive species, which provide information about the zebra mussel ecological impact,
- develop a method to differentiate unequivocally between both species for large samples.

TU 139 RA06P - Contaminated sediments in a changing environment HAW-Hamburg, Hamburg, Germany
Contamination of the Elbe estuary with particle bound compounds mostly (with the exception of TBT which had its source in the Hamburg Harbour) derives from "areas of risk" upstream of Hamburg such as the Czech Republic for organic contaminants like HCH, HCB, PCB, DDX and TBT, the tributary Mulde for As, Dioxins and HCH, and the Saale for a number of heavy metals like Cd, Hg, Cu, Zn, and Pb. The major transport of contaminants happens during periods of high water discharge, when historic contaminated soil or sediment in the catchments is released and transported downstream. For some compounds, the yearly load of particle bound contaminants is transported during a few days of a high water discharge (Heise, Krüger et al. 2008), up to now, the impact of contaminants on the quality of sediments in the estuary as a consequence of a high discharge event, however, has not been very well investigated. The results revealed by the Hamburg Port, the second largest port in Germany, discharges of up to 100 km upstream of the mouth of the river. Even though the river downstream of Hamburg has been maintained as a deep water navigation channel, 94 % of the area is ecologically protected by FFH and Birds directive. Some of the ecologically valuable freshwater wadden areas are close to Hamburg, and at the mouth of the Elbe the river widens to a large marine wadden area which is a feeding and nesting site of many bird and fish species. In order to assess the impact of a high water discharge situation on the sediment quality of ecologically important wadden areas, freshly deposited sediment (FDS) was sampled during normal and high water discharges at two freshwater mudflats close to Hamburg and at two places near the mouth of the Elbe close to the North Sea. Chemometric analyses were performed to gain insights on the sampling material. Elevated discharge did not lead to any significant increase of contamination fluxes may be recorded. Long term projections of regional climate models are variable and tend to predict more extreme rainfall and dry episodes but with a limited overall increase in river discharges and suspended particulate matter (SPM) loads to the North Sea. To study the possible impact of climate change on the quality of urban and coastal waters, a battery of in vitro biosays was applied to determine the toxicity profiles of environmental samples collected during climate change related events. SPM and sediment samples were collected in rivers from Norway, Sweden, Denmark, Germany, and The Netherlands during periods of dryness, flooding and extreme run-off. In addition, passive sampling was performed using silicone rubber sheets to evaluate the effects on dissolved contaminants. Extracts from SPM and passive samples were tested for respiratory toxicity (Microtox), dioxin-like activities, (anti)androgenic, (anti)estrogenic, and thyroid hormone-like activities, and for mutagenicity. Variable patterns of both increased or decreased toxicity in relation to other environmental factors were noted between the different rivers. For some compounds, toxic effects were observed (e.g. mutagenic effects on the bacteria Pseudomonas putida) in either sampling site. An extreme high water event in January 2011, however, led to an increase in contamination in freshwater as well as coastal mud flats. Especially high was the impact on the more upstream "Heuckenlock" which is of particular ecological importance. Ecotoxicologically, little effect could be measured and it needs to be clarified how biodegradable the historic contaminants still are after their transport downstream the Elbe River.

TU 141
Toxicity of suspended matter and sediments in relation to contaminant load and expected effects of climate change on European rivers
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4Climate change may not only affect river water discharges in terms of water quantity, but also in terms of water quality. During flooding periods, for instance, higher water discharges may cause a dilution of normal pollution levels, while on the other hand, higher discharges may also result in an increased mobilization of polluted sediments and in surface run-off from possibly polluted flood plains and inundated urban areas. During high water episodes huge increases of contaminant fluxes may be recorded. Long term projections of regional climate models are variable and tend to predict more extreme rainfall and dry episodes but with a limited overall increase in river discharges and suspended particulate matter (SPM) loads to the North Sea. To study the possible impact of climate change on the quality of urban and coastal waters, a battery of in vitro biosays was applied to determine the toxicity profiles of environmental samples collected during climate change related events. SPM and sediment samples were collected in rivers from Norway, Sweden, Denmark, Germany, and The Netherlands during periods of dryness, flooding and extreme run-off. In addition, passive sampling was performed using silicone rubber sheets to evaluate the effects on dissolved contaminants. Extracts from SPM and passive samples were tested for respiratory toxicity (Microtox), dioxin-like activities, (anti)androgenic, (anti)estrogenic, and thyroid hormone-like activities, and for mutagenicity. Variable patterns of both increased or decreased toxicity in relation to other environmental factors were noted between the different rivers. For some compounds, toxic effects were observed (e.g. mutagenic effects on the bacteria Pseudomonas putida) in either sampling site. An extreme high water event in January 2011, however, led to an increase in contamination in freshwater as well as coastal mud flats. Especially high was the impact on the more upstream "Heuckenlock" which is of particular ecological importance. Ecotoxicologically, little effect could be measured and it needs to be clarified how biodegradable the historic contaminants still are after their transport downstream the Elbe River.

TU 142
The effects of CO2 leakages from marine stable geological formations on the mobility and availability of metals
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Carbon dioxide capture and storage (CCS) in marine sub-seabed geological formations, is already contemplated in international conventions as a mitigation measure to reduce the concentration of CO2 in the atmosphere. Although this technology is currently considered to be technically feasible there is lack of information on the environmental impacts of CO2 injection. SO2 is a corrosive gas in the sediment, seepage pore water, sediment-water interface pH decreases in the water column. Increase in acidity will lead to several biogeochemical alterations that can result in detrimental effects in the ecosystem. Changes in the solubility, speciation, mobility and bioavailability of metals is expected as a direct result of pH decrease.

TU 143
Changes in the bioavailability of synthetic surfactants from river to marine sediments
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Aquatic systems are subjected to the influence of both urban and industrial wastewater discharges, which are among the main sources of organic contaminants. Due to their extensive use and applications surfactants, particularly high concentrations of surfactants can be found in wastewater. Once they reach water bodies, a significant fraction of these compounds can be detected in sediments due to their moderate sorption capacity. It is often assumed that surfactants are well preserved in the sedimentary column once anoxic depths are reached, which can happen within a few millimeters to centimeters depending on the aquatic system. In this work we have studied the diagenetic fate of the most commonly used surfactant:ulinol polyether (AEOs) and nonylphenol polyethoxylates (NPFOEs), both non-ionic, and linear alkybenzene sulfonates (LAS) and alkyl ethoxylates (AES), both anionic. Their concentrations in sediment and pore water were determined along the sedimentary column by sampling cores in three different aquatic systems from SW France: the salt marsh-estuary of the Bay of Cádiz, the middle stretch and the estuary of the Guadalete River, and the Bornos water body. These different distributions were observed according to the respective uses, production volumes and physicochemical properties of each surfactant. Levels of non-ionic (up to 12 mg/kg) were twice as high as to those for anionics near industrial areas and ports, whereas the opposite was found near urban wastewater discharge outlets (up to 18 mg/kg). The most remarkable result was that sulfophenyl carboxylic acids (SPC), LAS degradation products, were identified in anoxic pore water. However, this was observed only in marine, and, to a minor extent, estuarine sediments. No SPC were detected in sediments from freshwater systems. The presence of sulfite-reducing marine sediments (or absence in freshwater environments) seems to be strongly related to the degradation process. On the other hand, no changes in the average length of AEO and NPFOE ethoxylated chains were observed along sediment cores from any type of aquatic system, suggesting that their biodegradation is very limited in anoxic conditions. This may be directly related to the lower bioavailability of the ethoxylated chain, as their calculated sediment - pore water distribution coefficients showed much higher values when compared to those for most polar anionic surfactants (e.g., 535 ± 98 for NPFOEs vs. 11 ± 7 for AES).

TU 144
Historical trend and pollution assessment for selected trace elements in sediment cores from the Orbetello lagoon (Tuscany, Italy)
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Heavy metal enrichment in sediment may be due to both natural and anthropogenic contribution, which may be recognized by studying the concentration profiles along sediment cores. Contribution due to heavy metal in the sediment was carried out in the Orbetello lagoon (Tuscany, Italy). The lagoon is located within a volcanic district, characterized by hydrothermal ore deposits and natural positive geochemical anomalies for some heavy metals may occur. Consequently, the correct interpretation of natural and anthropogenic enrichment is a main issue for a correct environmental impact assessment. In this work, selected trace elements (As, Cd, Cu, Hg, Pb, and Zn) were determined in two different sediment cores (OR86 and OR96) collected from each element using local background concentrations. The determination of Cd, Cu, and Zn concentrations demonstrated in both the cores an upper anthropogenic enrichment referable to the last 20 years of the 20th century, while Pb contamination was found to begin earlier, during the first half of the last century. In spite of considerable EFs showed by trace elements, human impacts in the region, were still low to mid-nineteenth century. On the contrary, the significative enrichment recorded for As in lower levels of core OR96 were interpreted as the result of increased contribution from the Albegna River catchment, which was observed in present in ore deposits of Hg and Sn. The investigated heavy metals was Hg, may be considered element of concern, because the measured concentrations exceeded with few exception, the reference values used for sediment quality. For this reason, indication to investigate potential toxicity through studies on mercury bioavailability and ecotoxicological response arises from the present research.

TU 145
Rapid contact kinetic assay with Vibrio fischeri in the assessment of small streams sediments in the Czech Republic
P. Masner, J. Kuta, J. Klímová, L. Bláha
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Influence of feeding type and behaviour on whole sediment toxicity tests with the oligochaete Lumbriculus variegatus


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DESY, HASYLAB, Hamburg, Germany

Lumbriculus variegatus were exposed for 28 days to 0, 180, 560 and 1800 µg Zn g-1 sediment and received 2 feeding regimes. The partitioning of Zn between sediment, pore water and overlying water was enhanced by a 2-year equilibration period. Feeding regimes used in the toxicity tests were (i) food (Urtaica-seelosum) spiked into the sediment with no additional feeding during the exposure (SED+) and (ii) food feeding with TetraMin. We observed significant differences between both studied regions in the Czech Republic as well as between different sampling periods. Statistical analyses repeatedly revealed relatively poor correlations between chemical concentration data and toxicity, while total carbon (TOC) appeared to be related to observed toxic effects. The present study has successful application of the kinetic V. fisheri test in the effect-based monitoring of sediments demonstrating thus complementarity of both chemical and ecotoxicological data.

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TU 147

Toxicity of organic micropollutants in the Yangtze Three Gorges Reservoir - MICROTOX


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The Yangtze River Estuarine Reservoir constitutes part of former urban, industrial and agricultural areas. Consequently, substantial amounts of organic and inorganic pollutants were released into the reservoir. Beyond contaminants and nutrients enter the reservoir by runoff from adjacent agricultural areas as well as from sewage of industry, aquacultures and households. Periodical changes in water level cause flooding events and thereby a relocation of contaminated water, particulate matter and sediments used areas along the reservoirs shores.

In order to assess (i) possible links between molecular/biochemical responses and ecologically relevant effects, and (ii) if ecotoxicological effects might be related to adverse effects in fish from the field, sediment samples and fish were collected at selected locations and analyzed using a weight-of-evidence (WOE) approach with several lines of evidence.

The object of the poster presentation is to introduce the conceptual framework and to present first results of the ongoing study.

As previously addressed by Chapman & Hottel (2006) a variety of lines of evidence can be used in WOE studies. Briefly, (i) a comprehensive battery of acute (neutral red assay, Arthrobacter, fish embryo toxicity test and sediment contact assay with Danio rerio) and mechanism-specific bioassays (micronucleus assay and Ames test, EROD and β-AROM) characterize the toxicology and adverse health effects of pollutants, (ii) biomarkers, representing in situ parameters as well as (iii) persistent organic pollutants (PCB, PAH and PCDD/Fs), endocrine disrupting substances, linochemical parameters and the concentration of heavy metals will be taken at major cities as well as feeder rivers and their estuaries along the Yangtze Three Gorges Reservoir in order to characterize the state of contamination and the potential risk for human and wildlife. The monitoring strategy should help to observe the water body's quality and to serve as a basis to initiate if necessary counteractive measures.

References

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TU 149

Toxicity of persistent organic pollutants from Baltic Sea sediments in the fish embryo test and the EROD assay


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The Baltic Sea, one of the largest brackish water bodies in the world, is considered to be an exceptionally sensitive and endangered marine ecosystem. The average residence time of Baltic Sea water ranges between 25 and 50 years, thus facilitating the accumulation of pollutants in sediment. For a deeper insight into the ecotoxicological effects of persistent organic pollutants, sediments, biological test systems were applied with exhaustive PART extraction, were investigated for their dioxin-like activity and their embryotoxic impact on the development of zebrafish (Danio rerio) embryos. PLE extracts were considered to represent the entire toxic potential and the mild methanol/water extracts to contain rather bioavailable pollutants. Applied biotests were the fish embryo test in 96-well plates and the EROD assay with RTI W1 cells. Result extracts in contrast to the PLE extracts, which gave strong embryotoxic potential. Results on the EROD inducing potential indicated dioxin-like activity for all investigated sampling sites. PLE extracts showed clearly higher dioxin-like activity than mild methanol/water extracts. The study revealed an ecotoxicological burden for all Baltic Sea sediments. Especially the Eastern Gotland Basin, the São Francisco River and the Arctic Basin appeared highly polluted, but not for the methanol/water extracts, this pollution is not readily available for developing fish embryos. However, the ability of the mild extracts to resemble the bioavailable fraction might be limited. For a comprehensive ecotoxicological assessment it is necessary to thoroughly investigate the bioavailability and to identify the causative pollutants by chemical analyses.

TU 150

Identification of hazard factors and ecotoxicological risk studies of sediments from the Tietê River Basin (Brazil)

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The objective of this study as an example for a highly contaminated river system, is located in the most important economical center in Brazil, São Paulo state. The reservoirs constructed along its course are widely used providing drinking water, as water sources for agricultural irrigation and as recreation sites. Several approaches involving different test organisms, several endpoints and different sediment phases (liquid as extract and solid as freeze-dried samples) were followed under laboratory conditions. The results document that sediment samples are differentially polluted with contaminants which cause not only acute cytotoxicity, but also genotoxicity and AhR-mediated toxicity in fish cells, as well as embryo toxicity. Moreover, mutagenicity was recorded in situ in fish caught from the field. Since major toxicities were not detected in the sediment samples from this region were submitted to an effect-directed analysis, in order to identify key pollutants causing the high toxicity of sediments. Results indicated different sets of toxicants inducing genotoxicity and metabolic activation. Overall, genotoxicity could be mostly related to alkyls, polychlorinated biphenyls (PCBs), naphthalenes (PCNs) as well as medium to polar to polyaromatic hydrocarbon compounds (PAHs), whereas AhR-mediated toxicity could mostly be attributed to polycyclic aromatic hydrocarbons (PAHs). A comprehensive evaluation of the ecotoxicological situation of sediments from different sites of Tietê River Basin represents different approaches and in-depth knowledge for interpreting results. As exemplified for the Tietê River Basin, a battery of biosays applied in combination with chemical analyses and effect-directed analysis served suitable tools to function as early warning systems not only for sediment pollution, but also for hazards for the entire river system.
by means of chemical and ecotoxicological approach the toxicity of the sediments of this river, and their elutriates, in a region downstream Três Marias reservoir, in a site near a processing industrial plant of zinc. For this, a battery of three laboratory bioassays, using two cladoceran (Daphnia similis and Ceriodaphnia dubia) and one amphipod (Hyallela azteca) species, was used to assess the toxicity. 12 different metals (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) and the different geochemical sediment fractions were determined in the samples (first steps of the sequential extraction procedure) in order to establish the potential of the industrial plant. The effect of sediment metal contamination was assessed applying multivariate techniques.

The results show elevated concentrations of Zn, Cd and Pb that involve an important potential risk on the aquatic environment. The sediments affected by the industrial plant presented high levels of toxicity, although the hydrodynamic of the river plays an important role in the metal distribution and consequently in the toxicity of the sediments.

TU 152
Ecotoxicological assessment of sediments from rivers impacted by a petroleum refinery
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Ecotoxicological studies are important for evaluating the effects and mechanisms of action of toxic agents in different environments. Aquatic sediments have a high potential for accumulating pollutants. As pollutants may become available in certain environmental conditions, the sediments can also be a potential source of contamination to water bodies. This study was to evaluate the ecotoxicological potential of sediment samples from three rivers impacted by a petroleum refinery, by means of Neutral Red assay (cytotoxicity), EROD assay (CYP1A induction potential), the sediment contact with fish embryos (larvae toxicity) and chemical analyses, among other tests. From these samples from these rivers have also been investigated, these results must be associated, in order to obtain a comprehensive evaluation of the ecotoxicological situation of these water bodies. Final results will be presented.

TU 153
Seasonal variation of toxicity and genotoxicity of sediments samples of the Ensenada de la Paz, B.C.S. México
S. Sobrino-Figueroa, C. Cáceres-Martínez
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In this paper was carried out a qualitative assessment of toxic and genotoxic effects of sediment collected at 8 locations in the Ensenada de la Paz, B.C.S., in 2 contrasting seasons summer and winter of 2010, using a battery of biological tests with organisms of different trophic levels and the SOS-Chromotest microbioassay. Compounds with toxic and genotoxic effects were detected in 5 locations, located in the Canal de la Paz, near the termoelectric power station and the Pichilingue navy. LC50 and genotoxicity values were determined, indicating the presence of toxic compounds at sites near Punta Prieta and Cisnaden, sediment toxicity decreases with increasing distance from these points. This fact possibly indicates a contribution of harmful compounds probably from Punta Prieta and La Paz city to the Bay of La Paz. This type of analysis is essential to identify areas of risk in studies of environmental analysis.

TU 154
Toxic effects of sediments from a semi-arid coastal system, influenced by a gold ore mine
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More than 600,000 tonnes of pure arsenite are scattered in the area of the El Triunfo gold ore mine, in the southern most Baja California peninsula, México. The drainage basin, from which sediments were collected for this study, discharges into the Pacific Ocean, however, the river is dry most of the year, only occasionally filled during tropical hurricanes in summer. This coastal system receives a continuous contribution of toxic compounds due to mineral extraction in the area. The main goal of this study was to evaluate the toxicity of the sediments and the level of metal pollutants in order to determine the environmental risks. Heavy metals were determined in conjunction with sediment toxicity using Daphnia magna, Selenastrum capricornutum and seed-germination bioassay analysis. The Sediment Quality Triad (SQT) was used to identify areas in the system where sediment contamination may be linked to ecosystem degradation. A multivariate analysis was carried out and indicated that toxicity results were associated from modern to historical gold mining, which indicate that the stations nearest to the alteration zone were severely polluted. These results suggest that there are health risks to human populations and activities such as vegetable and cattle farming or rural touristic recreation in the area. In order to restore the alteration zone to healthy conditions a remediation program would be necessary.

TU 155
Bioassays in sediment assessment for investigating toxicological effects in the context of the WFD
M.H. Hémart, Y. Marneffe, R. Pirotte, V. Wrona, C. Chalon, P. Naport, V. Rollin, C. Corin, A. Classens
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An intense industrial activity has been taking place in Wallonia (Belgium) since the Middle Ages. The proximity to major waterways has enhanced the industrial development and this has led to an altered aquatic environment. Sediments are known for their ability to trap pollutants and release these substances years or even decades after a pollution has ended. In order to assess the current status of sediment pollution, bioassays can be used as suitable monitoring tools to answer the Water Framework Directive (WFD) requests and ascertain the causes of a water body failure to achieve the environmental objectives. They integrate all contaminants effects including additive and synergistic effects of unknown substances and breakdown products.

A sediment quality recent monitoring throughout the Walloon Region has been set up, taking into consideration physico-chemical parameters (including Prioritization List substances from the WFD) and eco-toxicological effects of collected environmental samples in a triad-type approach. Different species (Chironomus riparius, Heterocypris incongruens for whole samples, Vibrio fischeri, Pseudokirchneriella subcapitata and Brachionus calyciflorus for pore waters) are used in this approach because they provide different information due to a variety of exposure (ingestion, regurgitation) contact and because they represent different trophic levels (decomposer, producer or consumer). In total, the assessment was carried out at 10 stations and results mentioned that 7 stations were toxic to Vibrio fischeri, 5 to Pseudokirchneriella subcapitata and 4 to Brachionus calyciflorus. This toxicological analysis allows to identify areas of risk in studies of environmental analysis. The sediments isolated from the SQT indicate that the stations nearest to the alteration zone were severely polluted.

TU 156
Ecotoxicological assessment of contaminated rivers as a proxy for the water framework directive: an 1 micron air sampler
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Metallization of freshwater water bodies resulting from mining activities or deactivated mines is a common environmental problem in Portugal. Despite authorities recognized this problem, the establishment of a limit for the metals in the elutriates is only a first step in the remediation of these areas. In the Bragancinha mining area, which was a closed-down, which allows the Braga mine near the area of acid mine drainage phenomenon resulting from the oxidation in contact with wet. In opposite, Palladino mine never faced flooding events, which may explain the higher toxicity of the samples collected there. Our study highlights the usefulness of using an ecotoxicological approach to help the prioritization/scoring of the most critical areas impacted by deactivated mines.

TU 157
Assessment of effects of agro-chemicals use in ecological status of Kilombero Flood Plain, Ramsar site
E. Makasa1, D. Masblette2, J. Vacher1, H. Salm1, D. F. Angelis1
1University of Heidelberg, Heidelberg, Germany
2University of Aveiro, Aveiro, Portugal

Tanzania is rich in wetland resources with an estimated 10% of the land surface covered by significant freshwater wetlands providing essential ecosystem and livelihood support functions. Millions of people depend on wetlands (MNRT, 2003) for fishing, agriculture, livestock keeping and collection of a multitude of minor wetland products.

Assessment of effects of agro-chemicals use in ecological status of Kilombero Flood Plain, Ramsar site

TU 158
Seasonal variation of toxicity and genotoxicity of sediments of the Ensenada de la Paz, B.C.S. México
S. Sobrino-Figueroa, A.J. Marrodejo-Rodriguez1, V.R. Magalhães-Ortiz1
1University of Aveiro, Aveiro, Portugal

Seasonal variation of toxicity and genotoxicity of sediments samples of the Ensenada de la Paz, B.C.S. México
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TU 160
Ecotoxicological assessment of contaminated sediments from a gold mining area
M.H. Hémart, Y. Marneffe, R. Pirotte, V. Wrona, C. Chalon, P. Naport, V. Rollin, C. Corin, A. Classens
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E. Makasa1, D. Masblette2, J. Vacher1, H. Salm1, D. F. Angelis1
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Heavy metal pollution assessment in sediments of the Nenrut Bay, Turkey
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Surface sediments in the fraction <63 µm collected from eight stations in Nenrut Bay, Aegean Sea were analyzed for trace metals (Cd, Pb, Cu, Cr, and Zn) by inductively coupled plasma optical emission spectrometry (ICP-OES). The heavy metal concentrations in the marine environment were characterized by the affinity to bind to particulate matter and sorption processes. Adverse impacts on the benthic fauna are caused through chemical uptake and bioaccumulation of these contaminants. Ecologically relevant processes such as bioconcentration and baseline toxicity of POPs are mostly controlled by the chemical activity of the substance rather than by its total concentration in the sediments. To calculate chemical activity and thus to predict bioconcentration and the baseline toxic potential of a contaminant mixture we applied the freely dissolved concentrations of POPs in sediments to predict remobilization processes, as well as the sources of these elements.

TU 160
Triad approach for risk assessment of sediment and floodplain soil of a metal-polluted stream
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Due to historical emissions of hexachlorobenzene (HCB), the sediments of the ponds of Upper Rhine River in southern Germany remain highly contaminated (up to 1000 µg kg−1). HCB is a bioaccumulative and toxic persistent organic pollutant (POP). By reason of its strong sorptive characteristics, HCB is mainly bound to organic substances. However, geogenic clay minerals are also capable sorbents. Regarding the contaminated sediments, the spatial distribution of HCB is considerably more heterogeneous, then e.g. the distribution of polychlorinated biphenyls (PCBs). Further, maximum loads of HCB occur rather in larger particle fractions (63 - 2000 µm), than in smaller fractions (< 20 µm). Knowledge of the specific HCB-sorbtors in the different fractions is of importance, because their characteristics control sorption strength, which is an important factor for remediation as well as bioavailability of organic contaminants.

The mineral composition of sediments (measured by X-ray Diffraction, XRD) and its influence on the amount of sorption is presented, as well as composition and sorption capacities of the sediments (classified by particle size and content of organic matter) and Kd-values were determined in batch. Ecotoxicological tests were performed to test the mobility of HCB under changing environmental conditions. An ecotoxicological risk assessment for one floodplain soil that is heavily contaminated with HCB was carried out using the triad approach. Results indicate that the sediment is not a mobile source of HCB, but can be considered as a potential source when transported in water or sedimented on river bed. Sediment has been identified as major transporter and sequester of HCB. The variability in sediment characteristics is mainly attributed to their active properties and the characteristics of the rivers such as pH, redox, and high water discharge, which is investigated during sediment sampling. The study aims to investigate the effects of particle size on heavy metal concentration, study seasonal variation in the concentration of Cu, Ni, Pb and Zn in Ravensbourne River sediments. The influence of selection of reference materials between different compartments such as suspended sediment, bed sediment, bank sediment and the water column of Ravensbourne River. Sediment (bed and bank) and water samples were collected from Ravensbourne River over a period of 12 months. Sediment samples were tested for heavy metal contamination using the aquatic rega, sequential extraction using a 6 hour extraction technique proposed by Maiz et al. (1997).

TU 161
Hexachlorobenzene-contamination of sediments of the Upper Rhine River - identification of sorbents to assess bioavailability
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TU 162
River sediment sampling and environmental quality standards
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Surface sediments form an important part of the aquatic environment and provide habitat to benthic organisms. However, poor water quality has been linked to contaminated sediments which consist of complex materials transported within river or deposited on river bed. Sediment has been identified as major transporter and sequester of fluvial contaminants such as heavy metals, most especially the fine sediment. At present, there are no environmental quality standards (EQS) for bed sediments or fine contaminated sediments, and one of the major problems in establishing sediment EQS is the identification of the suitable sediment fraction to measure - the actively transported or previously deposited sediment. The variability in sediment characteristics is usually attributed to their active properties and the characteristics of the rivers such as pH, redox, and high water discharge, which is investigated during sediment sampling. The study aims to investigate the effects of particle size on heavy metal concentration, study seasonal variation in the concentration of Cu, Ni, Pb and Zn in Ravensbourne River sediments. The influence of selection of reference materials between different compartments such as suspended sediment, bed sediment, bank sediment and the water column of Ravensbourne River. Sediment (bed and bank) and water samples were collected from Ravensbourne River over a period of 12 months. Sediment samples were tested for heavy metal contamination using the aquatic rega, sequential extraction using a 6 hour extraction technique proposed by Maiz et al. (1997).

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The first sight into contamination of Polychlorinated biphenyls and Polybrominated diphenyl ethers in sediments of Tam Giang-Cau Hai Lagoon, Central Vietnam
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2Center for Marine Environmental Studies (CMES), Ehime University, Ehime, Japan
3Naoshima Institute for Environmental Studies, Tsukuba, Japan
4Rapid economic development and urbanization growth in Vietnam have made this country become an important subject for extensive studied dealing with environmental pollution recently. So far, there has been no information on PBDEs contamination in Central Vietnam and the information about PCBs sources and levels is limited. Tam Giang-Cau Hai Lagoon lies along the coastal line of Thua Thien-Hue Province, Vietnam. Due to recent industrial and economical growth in this region, a large amount of untreated industrial and domestic wastewater is discharged to this river – lagoon system which can cause adverse environmental consequences and increased health risk for local communities. In this study, the contamination status, distribution and congener profiles of PCBs and PBDEs in surficial sediment of Tam Giang-Cau Hai Lagoon were investigated. PCBs and PBDEs were detected in all sediment samples with mean concentrations of 0.19 and 0.56 ng/g dw, respectively. The highest concentrations of both contaminants were observed downstream at the river mouths, indicating the river flow as a main source of pollutants into the lagoon system. The similarity of PCB congener distributions in sediments suggest the presence of a unique source over the entire study area, probably connected to local use of old electrical equipments containing
low chlorinated PCB formulations. Atmospheric transport & post depositional processes might modify to some degree the fingerprint of PCB inputs to the environment favouring the predominance of 3, 4 & 5 chlorinated congeners. The PBDEs profile in sediment was relatively uniform, with decBDE as the dominant congener, followed by nona- and octaBDEs, indicating DecBDE as the major technical mixture in consumer products in this region. In general, the levels of PCBs and PBDEs in lagoon sediments were lower than those reported in other river and estuarine developed countries. This may reflect a limited usage of PCBs and PBDEs in this region. Comparing to sediment quality guidelines currently used around the world, present sediment concentration of PCBs and phenolic compounds are far below the Chinese drinking water guideline values. The concentrations of PCBs and phenolic compounds in the samples were in the same order of magnitude of those reported on present levels in European and American countries, which show that potential risk are negligible due to PCBs and phenolic compounds contamination in these samples.

TU 167

Chemical and biological assessment of metal pollution in the Urdaibai Reserve of the Biosphere (UNESCO) using oysters as sentinels

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2Centre for Functional Ecology, Coimbra, Portugal
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Measurement of trace metal is interesting in the ecosystems of the Urdaibai Biosphere Reserve, within the sustainable management of the Gulf of Mexico. The capacity of the oyster (Ostrea edulis) to bioaccumulate metals has been used in several biological monitoring studies in order to assess the environmental quality of coastal and marine sites. The aim of this study was to use this species as a sentinel to assess the distribution of metals in the surface sediments of the Urdaibai Biosphere Reserve. The concentration of 14 elements (Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn, V and Zn) were determined using ICP-OES/MS and ICP-MS. The concentrations of these elements were compared to the sediment quality guidelines currently in use in the Basque Country and the results were used to assess the environmental quality of the Urdaibai Biosphere Reserve. The results show that the concentrations of metals in the surface sediments of the Urdaibai Biosphere Reserve are below the sediment quality guidelines in most cases, indicating that the biosphere reserve is in good environmental condition. However, there were some areas with high concentrations of metals, indicating the need for further investigation and monitoring.

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Environmental monitoring and assessment of Terminos Lagoon, Mexico

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Terminos Lagoon is a lagoon located in the state of Veracruz, Mexico. It is a shallow body of water surrounded by mangrove forests and is an important site for the conservation of local flora and fauna. The lagoon is an important habitat for a variety of birds and other wildlife, and is an important ecosystem for the local economy. Despite its ecological and economic importance, the lagoon is facing a number of environmental challenges, including pollution from run-off and coastal development, and the introduction of exotic species. This study aimed to assess the environmental quality of the Terminos Lagoon and to identify areas of concern. The study was conducted using a combination of field surveys, laboratory analyses, and modeling. The results show that the lagoon is under threat from a number of environmental stressors, including pollution from run-off and coastal development, and the introduction of exotic species. The study recommended a range of actions to improve the environmental quality of the lagoon, including the development of a comprehensive environmental management plan, the implementation of pollution control measures, and the identification and protection of key areas of the lagoon.
Monitoring of sediments genotoxicity of the Yucateco lagoon, Tabasco, Mexico

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The lagoon system is located in southeastern of the Mexican Republic in the Tabasco state. This system receives a continuous contribution of toxic compounds due to hydrocarbons extraction and industrial activity carried out in the zone. Since the system has been reported mass mortality events of fish and shellfish, in this research, an evaluation of the genotoxic effects of sediments over a period of 3 years was carried out to establish the state of ecosystem health. Sampling were made annually during the dry and rainy season (November to April and May to October) and were obtained from three stations located in the external, intermediate and internal areas, respectively. In general can mention that the number of sediment samples with genotoxic effects decreased from 2003 to 2007. In 2003 was detected genotoxic compounds in 5 sampling stations, while in 2007 this effect was observed only in 3 locations. Critical areas are the stations 1, 2 and 3 located in the northern and central part of the system, the genotoxic effects decreases with increasing distance from these points in the direction of outlet the river Tonalá. The critical pollutants likely to cause genotoxicity were the HAP's, PCBs (polychlorinated biphenyls) and metals vanadium and nickel. The utility of this type of analysis is essential to identify areas of risk in studies of environmental analysis.

Bang for buck: the challenge of choosing environmental indicators of anthropogenic impacts in estuaries

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Ecological assessments over large spatial scales require that anthropogenic impacts be distinguishable above natural variation, and that the monitoring tools implemented maximise detection impact for a relatively low cost. For three heavily modified and four relatively ‘pristine’ estuaries (disturbance category), chemical indicators (metals and PAHs) of anthropogenic stress were measured in benthic sediments, suspended sediments and deployed oysters, together with other environmental variables. The differing ability of these data sets to explain the variances observed in infaunal and hard-substrate invertebrate communities were compared. Univariate analyses were useful for comparing contaminant loads between different monitoring tools and identified the strongest correlations between benthic and suspended sediments. However, multivariate analyses are necessary to distinguish ecological response to anthropogenic stressors from environmental “noise” over a large spatial scale and to identify sites that were being impacted by contaminants. These analyses provide evidence that suspended sediments are a useful alternative monitoring tool to detect potential anthropogenic impacts on benthic organisms.

Metal availability and toxicity in estuarine systems in relation to chemical speciation and biological diversity

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The estuarine environment is a very dynamic system characterized by strong physical and chemical gradients and an enormous diversity in flora and fauna which undergoes major transitions moving from the freshwater to the marine areas. Along these gradients metal concentrations and speciation undergo important changes which also have an impact on the distribution and abundance of the biota. Parallel to this are changes in the physiological organisation of the biological species living in this gradient as they adjust themselves to the changes in ion composition and osmotic pressure of the external environment in order to maintain homeostasis balance. The effect of changes in salinity on metal speciation, metal bioavailability and metal toxicity was studied under controlled conditions in a series of structurally and functionally different estuarine environments where freshwater contribute to the water column. The results were discussed and compared with information available from databases and literature to explore whether general concepts such as the free metal ion activity model and major ion competition effects used to describe metal uptake and toxicity remain valid across estuarine gradients and biodiversity. Using a dynamic modelling approach the effects of changes in physical and chemical properties on metal speciation and toxicity were simulated across species diversity. For some metals, such as cadmium, where a strong inorganic metal speciation effect is observed a species sensitivity distribution analysis shows a decrease in metal toxicity moving from freshwater to higher salinity environments. However, it is not the case for a metal such as copper and in any case the effect is considerably less that what would be expected on the basis of the changes in salinity and metal ion activity. The results of these observations are further discussed by way of a case study of a site where a strong inorganic metal speciation effect is observed.

Ecological risk management at a site adjacent to an area of special scientific interest

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The estuarine environment is a very dynamic system characterized by strong physical and chemical gradients and an enormous diversity in flora and fauna which undergoes major transitions moving from the freshwater to the marine areas. Along these gradients metal concentrations and speciation undergo important changes which also have an impact on the distribution and abundance of the biota. Parallel to this are changes in the physiological organisation of the biological species living in this gradient as they adjust themselves to the changes in ion composition and osmotic pressure of the external environment in order to maintain homeostasis balance. The effect of changes in salinity on metal speciation, metal bioavailability and metal toxicity was studied under controlled conditions in a series of structurally and functionally different estuarine environments where freshwater contribute to the water column. The results were discussed and compared with information available from databases and literature to explore whether general concepts such as the free metal ion activity model and major ion competition effects used to describe metal uptake and toxicity remain valid across estuarine gradients and biodiversity. Using a dynamic modelling approach the effects of changes in physical and chemical properties on metal speciation and toxicity were simulated across species diversity. For some metals, such as cadmium, where a strong inorganic metal speciation effect is observed a species sensitivity distribution analysis shows a decrease in metal toxicity moving from freshwater to higher salinity environments. However, it is not the case for a metal such as copper and in any case the effect is considerably less that what would be expected on the basis of the changes in salinity and metal ion activity. The results of these observations are further discussed by way of a case study of a site where a strong inorganic metal speciation effect is observed.

Integrated estuarine Modelling to support watershed management for salmon and dyes inlets, Puget Sound, WA, USA

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An integrated, data-supported modelling system was developed to simulate the release, transport, and fate of PCP loading from watershed sources pointing to stream mouths, stormwater outfalls, waste water treatment plant discharges, and shoreline drainage areas. A total of 20 simulation scenarios were run to verify model performance, assess sensitivity and uncertainty, and provide results needed to establish management goals for the watershed. Model results indicated that for marine waters near the mouth of the river, PCP was found to be important for pheromone signaling and part of a general model of chemicals (metals, PAHs) which are transported through the system. The integrated watershed-receiving water model performed very well. The model was able to recreate a range of dynamic loading within the inlets, from large-scale storm events with high flow conditions to dry, low-flow conditions during the summer months. The integrated watershed modeling and modeling approach to water quality management is assisting the development of management plans worthy of stakeholder acceptance, helping to achieve reductions in FC loading, and resulting in improvements to the environmental quality of the inlets.
group in freshwater fish, 14.4% in the 8 ppt and no mortality in the 17 ppt salinity treatment groups indicating protection by hypersaline conditions from acute lethality. However, significant differences were not observed in the 8 ppt salinity group treatments indicating protection by hypersaline conditions from acute lethality. Several laboratories from academia, industries and government participated in an interlaboratory ring test organized by the German Federal Institute of Hydrology. The sediment contact test with Myriophyllum aquaticum (ISO/CD 16191): first results of an international ring test

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Sediment contact test with Myriophyllum aquaticum (ISO/CD 16191): first results of an international ring test

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Although dicotyledonous macrophytes are not part of the risk assessment of sediments or dredged material, the growth of Myriophyllum aquaticum in a test sample is compared with its growth in the control sample. Phytotoxic effects can be quantified as growth inhibition (%) relative to the control growth.

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Genotoxicity and ecologically relevant data from laboratory tests: recovery potential of Lemna minor

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The goal of ecological risk assessments for chemicals typically is to protect the status of populations. Still, the assessments are usually conducted at the level of the individual, so the results of laboratory testing need to be translated into predictions of population status. Extrapolation from laboratory tests has many limitations, among which is the fact that species differ not only in toxicological sensitivity but their potential for recovery from adverse effects. In the study on sensitivity and recovery potential after exposure to atrazine (Teodorovic et al., 2006) two test groups were used. The ability of test species to recover following sub-lethal exposure to herbicides was examined using the sulfonylurea herbicide chlorimuron ethyl. Eight wetland and nine upland species were tested with eight doses of chlorimuron (+ controls) ranging from 1 to 107% of the recommended label rate.
of 9 g ha-1. For each species, two sets of plants were grown: total aboveground plant biomass was assessed at four weeks after exposure for the ST plants (first set) and again for the LT plants (second set) when the controls began natural senescence (7-23.5 weeks post-spray depending on species). To assess recovery over time, measurements of maximum plant height were taken weekly for all species using the LT plants. Reproductive parameters were also recorded for 13 species. Inhibition concentrations (IC50) were determined for ST and LT biomass and for LT reproductive parameters. LT biomass was found to be the most sensitive measure of IC50 for nine species and LT reproduction for three; IC50s could not be predicted for five species (no effect). Delays in flowering time were apparent for several species. Twelve species had early delays in growth as compared to the controls at doses of 7.4% or less, two species were affected at higher doses (14.5 or 55%) while three species (all grasses) had no reductions in height. No species were affected at doses of 7.4% or less, three fully recovered within an avg. 6.7 weeks post-spray, six recovered up to doses of 14.5% (avg. 6.5 weeks) and three up to doses of 28.2% (avg. 12.3 weeks). Though an overall trend toward recovery was also noted for reproductive parameters (4 spp. recovered, 3 remained the same, 3 unaffected at any dose), rates of recovery did not follow those observed for height. Delayed growth and flowering was observed at doses less than known drift rates. Rates of recovery were assessed on the LT series. While recovery did occur in this greenhouse experiment, it may be suppressed in natural conditions where more resistant species (e.g. grasses) may grow faster and out-compete susceptible ones for light or resources. Such events may alter the natural species composition in exposed areas. This community approach is not considered in risk assessment.

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Effects of agrochemicals on macrophyte community structure and quality in a Canadian agricultural watershed
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Macrophyte communities inhabit rivers and lakes in temperate regions. Unfortunately, macrophyte diversity, particularly diversity of submerged species, appears to have declined in some North American and European ecosystems during the last century. In agricultural watersheds, streams are intimately connected with croplands and the health of macrophyte communities may be compromised by increased levels of disturbance and exposure to agrochemicals such as nutrients and herbicides in areas of high-intensity agriculture. The project was undertaken in an area identified as the South Nation Watershed located throughout the South Nation River watershed, a sparsely populated agricultural watershed comprising 3919 km² in Eastern Ontario, Canada. The sites varied in terms of their contamination with nitrate and the commonly used herbicide atrazine, with mean spring maximum in-stream concentrations of 6600 μg/L and 1.56 μg/L respectively. In-stream concentrations of nitrate and atrazine were strongly correlated. Over 200 macrophyte species were identified along stream banks and through the stream channels. Sites surrounded by high levels of agriculture tended to have a higher percentage of non-native species, suggesting that native species are less able to grow across the watershed. A decline in the quality and conservation values of macrophyte communities was observed along a gradient of nitrate contamination.

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The effect of five sulfonyl-urea herbicides to aquatic macrophytes - implications of results from outdoor growth inhibition studies for the risk assessment
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Ten sulfonyl-urea herbicides were applied to the pot experiment in order to assess their effects on the growth of aquatic macrophytes. Of these, only azoxystrobin (AZX), a compound approved for use on almost 100 different crops, had a pronounced impact. Its mechanism of toxicity is well understood in fungi but it effects in plants are not well understood. Strobilurin fungicides constitute a relatively new fungicide class developed from natural fungicidal derivatives and the great impact of them on agriculture is reflected by the large amount of data obtained enables refined risk assessments with low assessment factors based on the lowest EC50 as well as probabilistic approaches using a hazard concentration (HC5).

TU 187

Ecological functions of plant growth regulators in the stability of agrocenosis
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Farmers often use growth regulators in agriculture. "Active substances" (growth regulators) are biologically active compounds. Their use is intended to improve the activity of some agrocenosis elements (e.g., the element pool (As, Cu, Pb and Zn)) and to identify a most sensitive species. Lemna is the standard aquatic test organism and among the most sensitive species to herbicides. Multiple species data provide arguments to lower the assessment factor due to increased realism and lower uncertainty. The example of sulfonyl-urea herbicides (SU) outlined here shows that herbicides of the same action can be more or less sensitive in different natural conditions.

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Biochemical responses in the aquatic macrophyte Myriophyllum quinseei exposed to the strobilurin fungicide azoxystrobin
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Strobilurin fungicides cause a very reduced net primary production and a large class developed from natural fungicidal derivatives. The main goal of this study was to assess the effect of azoxystrobin exposure on the growth, reproduction, and oxidative stress in Myriophyllum quinseei. We observed an increase in chloroplast and chlorophyll concentrations in plant tissues, the activity of Guaiacol Peroxidase (POD), Glutathione S-Transferase (GST), Catalase (CAT), and the chlorophyll a and b contents were tested. Plants were exposed to 0 (negative control), 0.1; 1; 10; 30 and 100 μg L-1 of AZX for 24 hours. All the parameters were measured spectrophotometrically. Inhibition of CAT and POD at 30 μg L-1 was observed (p<0.05), showing the stress conditions expected for any Concet. GST and POD activities showed significant differences in all treatments (p<0.05). Although there was no change in the chlorophyll a/chlorophyll b ratio among treatments an increase of total content of both pigments at 100 μg L-1 with respect to control was observed (p<0.05). Our results show the importance of using biomarkers of different sensitivity, in the evaluation of potential negative effects of emerging agrochemicals in the environment, being a more detailed study highly recommended.

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Impact on surface water, stream sediments and macrofreshwater from the Aljustrel mining area (Portugal)
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The main environmental problem in the vicinity of Aljustrel mining area, as in other massive sulphide deposits of the Iberian Pyrite Belt, comes from the tailings deposited at the site which contain large concentrations of elements (namely As, Cu, Pb and Zn) and can react with oxygen and water producing acid mine drainage (AMD). The AMD is usually discharged in the downstream areas, affecting surface water, groundwater and stream sediments. That impact is evident at Água Farta stream, which receives the AMD from the Aljustrel mining area, tributary of the Ribeira de Aljustrel stream. The aim of the study was: a) to evaluate the extent of pollution in stream sediments and atsediment at Água Farta and at Rosso, and b) to assess the potential of the macrophyte Scirpus bulbosus, collected along the stream banks, for phytoremediation of AMD. Several sampling sites were selected at Água Farta and Rosso stream and downstream from the confluence of the Água Farta stream. Water quality was assessed considering physico-chemical characteristics and ecotoxicological parameters. Sediment samples were analyzed considering general physico-chemical characteristics and a sequential extraction procedure was used to quantitatively assess trace element pool (As, Cu, Pb and Zn). Scirpus bulbosus samples were analyzed in order to assess trace element concentrations in the aboveground plant material.
Both surface water and sediments were extremely acidic at Água Forte stream, with pH values <2.92 for surface water and <3.12 for sediment samples. Surface water at Roxo stream presented pH values >7.26, apparently not affected by the confluence of the Água Forte stream, but the same was not true when considering the pH and other characteristics of the sediments. Ecotoxicological biossays (luminescence inhibition of Vibrio fischeri and 48-h immobilization/mortality assay with Daphnia magna) were conducted to determine the water quality. Although high As, Cu, Pb and Zn concentrations were found at Água Forte stream, the same was not true in Roxo stream. Trace element content in water, sediments and Scirpus holoschoenus samples was analyzed and correlated in order to ascertain if this particular macrophyte, which seems adapted to the extreme conditions found at Água Forte stream, can be potentially used for metal removal in constructed wetlands to treat AMD. 

**TU 191**

**Species-specific responses to zinc in Lemnaeae: zinc storage and impacts on photosynthesis along frond developmental gradients**

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Lemnaeae are used to represent all macrophytes in standard aquatic toxicity testing, with protocols allowing for the use of different species. Recently, differential sensitivity in Lemnaeae species to zinc, based growth and chlorophyll-a fluorescence parameters, has been reported. Chlorophyll-a fluorescence is an effective tool for sensing and monitoring the effect of contaminants on the photosynthetic apparatus of aquatic plants. This study investigated if and how the concentration of zinc influences the photosynthetic apparatus of aquatic plants, with a focus on morphology, physiology and the antioxidative defence system. The hypothesis is that the concentration of zinc influences the photosynthetic efficiency, as well as zinc accumulation and storage, particularly in the species Lemnaeae. The maximum quantum efficiency of photosystem II (Fv/Fm) was measured in mature and young fronds as well as a developmental gradient with young fronds within the same species. Results showed that the effect of zinc accumulation was not significant in young fronds and that changes in the soluble and bound forms were observed after seven days. L. punctata and L. minor accumulated more zinc in their tissues than L. gibba. Partitioning of zinc in L. minor was notably different from the other two species, zinc was stored mainly in roots in the bound fraction, Fv/Fm and Y(II) in young fronds were more severely impacted by zinc than in mature fronds in all species. However, younger proximal sections of L. punctata fronds were more impacted than older distal frond sections compared to more homogenous impact in single Lemna fronds. Single colony, time-point or leaf-zone analyses may not show the full biological picture of the impact of a toxicant, especially not in L. punctata. Therefore, selection of Lemnaeae species and developmental stages of fronds should be given careful consideration when using chlorophyll-a fluorescence for assessing the impact of a toxicant in Lemnaeae.
The toxicity of the iron nanomaterials to aquatic and terrestrial plants
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Zero-valent iron (Fe0) as well as ferrate (Fe(VI), Fe(IV)) nanoparticles are well known especially for the ability to degrade a wide range of contaminants in both soils and ground waters. Although these materials are widely used in remediation processes across the world, almost no data are published about their ecotoxicological properties. This study aims to provide the first information about their effects on both aquatic (Leucaena leucocephala) and terrestrial (Sinapis alba) macrophytes. Our experiments show large differences in toxicity between tested species as well as oxidation state of nanomaterials. The higher an oxidation state the more pronounced toxicity and the higher EC50 values have been found, following the order as Fe(VI) > Fe0 > Fe2.

The abundance of P. vulgaris and several heavy metals at the same time made the plant our first choice for rhizofiltration methodology development.

Genetic variation in metal-tolerant Silene vulgaris clones
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Silene vulgaris is a facultative metallophyte with multiple co-tolerance to heavy metals, capable of colonizing contaminated sites due to its fast and vigorous growth by producing seeds and rhizomes. In order to evaluate its potential for the purpose of phytoremediation, genetic approaches are required to uncover the allelic variation at both Mendelian and quantitative genes. Analytical tools based on the identification of useful genetic diversity are needed for plant genotype selection aimed at enhancing remediation efficiency. This study considers the genetic relationships among different S. vulgaris clones. Fourteen clones from ten populations collected from Madrid (Spain) were analysed using chloroplast DNA (cpDNA) markers. Ten primers pairs chloroplast SSR loci were tested and 3 of the 10 primer pairs revealed the existence of intraspecific length polymorphisms. The intergenic spacer between the trnH and psbA genes was amplifi ed with the primers trnH (CUG) and psbA. All PCRs produced a major robust band per primer pair in all samples studied. Two size variants were detected for ccmP (120 and 121pb), two for ccmP4 (113 and 114pb), five for ccmP2 (190, 191, 192,193 and 198pb) and six for trnL/hps4 (328, 338, 349, 354, 355 and 357pb). Regeneration of polymorphism, ten unique cpDNA haplotypes were identified and seven of them were private, being found in only one population. Despite of limited number of clones examined, a considerable intraspecific polymorphism was detected and can be explained by S. vulgaris out-crossing breeding system. Conservation of genetic resources in this allogamous species requires maintenance of the genetic diversity within each clone to avoid inbreeding depression and loss of rare alleles.

Biosorption of Pb by Chlorella vulgaris (Chlorophyceae) at laboratory scale
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Chlorella vulgaris is a facultative metallophyte with multiple tolerance and co-tolerance to heavy metals, capable of colonizing contaminated sites due to its fast and vigorous growth by producing seeds and rhizomes. In order to evaluate its potential for the purpose of phytoremediation, genetic approaches are required to uncover the allelic variation at both Mendelian and quantitative genes. Analytical tools based on the identification of useful genetic diversity are needed for plant genotype selection aimed at enhancing remediation efficiency. This study considers the genetic relationships among different S. vulgaris clones. Fourteen clones from ten populations collected from Madrid (Spain) were analysed using chloroplast DNA (cpDNA) markers. Ten primers pairs chloroplast SSR loci were tested and 3 of the 10 primer pairs revealed the existence of intraspecific length polymorphisms. The intergenic spacer between the trnH and psbA genes was amplifi ed with the primers trnH (CUG) and psbA. All PCRs produced a major robust band per primer pair in all samples studied. Two size variants were detected for ccmP (120 and 121pb), two for ccmP4 (113 and 114pb), five for ccmP2 (190, 191, 192,193 and 198pb) and six for trnL/hps4 (328, 338, 349, 354, 355 and 357pb). Regeneration of polymorphism, ten unique cpDNA haplotypes were identified and seven of them were private, being found in only one population. Despite of limited number of clones examined, a considerable intraspecific polymorphism was detected and can be explained by S. vulgaris out-crossing breeding system. Conservation of genetic resources in this allogamous species requires maintenance of the genetic diversity within each clone to avoid inbreeding depression and loss of rare alleles.

Bioaccumulation and distribution of selected metals in the macrophyte Ceratophyllum demersum, in a polluted South African river
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Selected macrophytes Ceratophyllum demersum, introduced to the Diep River, Cape Town, South Africa. The Diep River passes various possible sources of metal pollution before reaching the Atlantic Ocean. Previous studies on this river have shown high levels of metal contamination, however, studies on metal bioaccumulation in aquatic biota such as plants are limited. Plants were obtained from a reference site and placed in baskets at two sites along the river (one upstream and one downstream from urban pollution sources). Plants, sediment and water were collected every fortnight for twelve weeks. Samples were analysed for aluminium, iron, zinc and copper using nitric acid digestion and analyzed by means of ICP-MS. The results generally indicated that plants collected upstream had significantly higher concentrations of all four metals, compared to those collected downstream, possibly due to intensive agricultural activities upstream and the filtering effect of aquatic macrophytes. Also, C. demersum generally rapidly bioaccumulated higher concentrations of metals in its leaves, compared to stems, possibly as a result of having a greater surface area for uptake. Additionally, metal-laden leaves may be used as a detoxification mechanism. In conclusion, it is clear that the metals in the Diep River are bioavailable for uptake in aquatic biota. Also, C. demersum may possibly be used in biomonitoring programmes. However, further research is necessary to investigate the toxic effects of the metals and how it relates to metal exposure.

Selenium pathway in water moss Fontinalis antipyretica
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Water mosses are good indicator of polluted watersources. We investigated the uptake of Se in widely distributed truly water moss Fontinalis antipyretica in its natural environment. Nine locations in Notranjska region, Slovenia, with different land use in the catchment, were sampled. Samples of water and moss were taken in all four seasons
during years 2009, 2010 and 2011. The concentration of Se in water from all locations did not exceed 0.2 µg L⁻¹. Fontinalis antipyretica took up Se in the range between 343 - 3039 ng Se g⁻¹ (on dry matter basis, DM). The Se content varied, depending on the location and season. The highest content of Se was measured in the stream of Zerovichnica that flows through agricultural area with pastoral farming, the value being 3039 ± 170 ng Se g⁻¹ (DM). The amount of insoluble Se compounds after enzymatic hydrolysis using Protease (XIV) was around 75%. In soluble Se compounds only traces of Se (IV) and Se (VI) were found. No other Se compounds (SeMeSeCys, SeMet) were detected.

TU 202

The major organic matter contribution of marsh salt water to coastal wetlands ecosystems: a case study in Jiangsu, China
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Stable isotope techniques and different calculation models were used to analyse the potential sources of sediments organic matter in the core area of primary wetland and to calculate the contribution of salt marsh vegetation. By using multiple resources linear mixing model and the Euclidean distance model, we can conclude that micro-algae and Spartina alterniflora are the main contributors, under the assumption that the δ¹³C value of micro-algae is -23%, the average contribution rate of micro-algae is 40%, which is the main contributor to the salt marsh wetland ecosystems in northern Jiangsu. The contribution rate of Spartina alterniflora to its marsh is 56% and to the intertidal is 75%. By comparing the two quantification models, we can find that the Euclidean distance model would overestimate the contribution of incidental resources and average the resource of high contribution rate.

TU 203

Genotoxicity study on Vicia faba L. grown on natural and spiked contaminated soils
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Contaminant bioavailability and toxicity varies among soil types and may depend on soil properties and on contaminants characteristics. Metals contamination could have different potential risks depending not only on the total content but on their bioavailability. Operationally, metal bioavailability is often evaluated by chemical extractions characterizing different chemical forms. The use of plant bioassay may be an amenable tool to screen the phytotoxicity of contaminated soils. This is the first time that the procedure was performed on different soils contaminated by Boron (B) and lead (Pb). In this work different soils contaminated by Boron (B) were studied in relation to plant bioavailability and toxicity. B is an essential micronutrient for plants and generally is absorbed from soil in the form of boric acid. Previous studies demonstrated that both excess or deficiency of B can affect normal plant development. Naturally polluted soils and artificially contaminated soils showed the same pattern of phytotoxicity and genotoxicity in Vicia faba. Classical end points might be masked by abundance of nutrients and nutrients are likely to be available from the artificial contamination. Mitotic Index and Chromosomal Abnormalities (CAs) were found in control samples. In the artificially polluted soils is possible to find more abnormalities in mitosis than in control samples indicating an overestimation of water toxicity. Our results revealed that different sources of pollution (control soils, artificially spiked samples) showed the same pattern of phytotoxicity and genotoxicity in Vicia faba. It is interesting to stress that bioavailability of B, and its content in the different plant organs, resulted higher in artificially spiked soils when compared with the same B concentrations in natural soils. A close correlation between high B concentration in roots and major level of cytogenetic defects was found. These results confirmed plant genotoxicity of B polluted soils and showed clear evidences between soil properties, B bioavailability and phytotoxicity.

TU 204

Acute and chronic in vitro bioassays vs. autochthonous plants used as sentinel: comparing genetic biomarkers as tools in ecological risk assessment in Del Plata basin
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Del Plata basin is the fifth largest river basin in the world, its area covers four million km² approximately and contains a great variety of natural environments and resources; these resources are shared by Bolivia, Paraguay, Brasil, Uruguay and Argentina.

The lack of wastewater treatments from 1000 million inhabitants is the main source of chemical and biological pollution that impact on all tributaries and affects negatively to the aquatic environment.

Samples of surface river water from along 1200 km of river coast were analyzed according acute water quality indexes (WQI) were calculated Value were adjusted to Argentine guidelines and sampling sites were site quality, being Excellent (95-100) 11%, good (80-94) 21%, Fair (65-89) 26%, Marginal (45-64) 26% and Poor (0-44) 20%.

In this work different soils contaminated by Boron (B) were studied in relation to plant bioavailability and toxicity. B is an essential micronutrient for plants and generally it is absorbed from soil in the form of boric acid. Previous studies demonstrated that both excess or deficiency of B can affect normal plant development. Naturally polluted soils and artificially contaminated soils showed the same pattern of phytotoxicity and genotoxicity in Vicia faba. Classical end points might be masked by abundance of nutrients and nutrients are likely to be available from the artificial contamination. Mitotic Index and Chromosomal Abnormalities (CAs) were found in control samples. In the artificially polluted soils is possible to find more abnormalities in mitosis than in control samples indicating an overestimation of water toxicity. Our results revealed that different sources of pollution (control soils, artificially spiked samples) showed the same pattern of phytotoxicity and genotoxicity in Vicia faba. It is interesting to stress that bioavailability of B, and its content in the different plant organs, resulted higher in artificially spiked soils when compared with the same B concentrations in natural soils. A close correlation between high B concentration in roots and major level of cytogenetic defects was found. These results confirmed plant genotoxicity of B polluted soils and showed clear evidences between soil properties, B bioavailability and phytotoxicity.

TU 205

Can we possibly derive environmental quality benchmarks for chemical mixtures?
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In recent years, there are increasing concerns in the aquatic environment. For example, many anti fouling biocide residues are often detected as a cocktail in water and sediment samples collected from coastal environments. Based on literature reviewed of studies on the combined ecotoxicity of anti fouling biocides, we found that both additive and synergistic effects together account for 80% of all cases in which about 35% cases are synergistic. To allow more accurate risk assessment of concurrently occurring chemicals, there is a need to develop environmental quality benchmarks (EQBs) for their mixtures. In this presentation, I will introduce and informal description of deriving EQBs for chemical mixtures. First, if all components in a chemical mixture are known to share a similar toxic mode of action, we can assume that the combined toxicity of the mixture would follow a simple concentration addition model, and the concept of toxic equivalency quotient (TEQ) could be applied to derive the EQBs based on concentration of each chemical. If the mixtures contain chemicals with different toxic modes of action, it is possible to use the impulse response models for the multidimensional species sensitivity distribution (m-SSD) approach. Here, I use binary mixtures of copper (Cu) and zinc pyrithione (ZnPPT) as an example to illustrate the m-SSD method. We first conducted standard acute toxicity tests with an array of marine organisms for each chemical alone, and for their mixtures. The Cu-ZnPPT mixtures showed a strong synergistic toxic effect to all test organisms. By utilizing the toxicity data, we are able to construct a two-dimensional SSD in form of a response surface, from which we can derive any specific hazardous concentration (i.e., EQB) for the two compounds. This novel method can be potentially applicable to a more complex mixture by employing non-parametric response surface models. Third, I will highlight the field-based SSD approach, which is improved with the quantile regression method, can be used to derive sediment quality guidelines for any target chemical with consideration of the presence of chemical mixtures and biological interaction. Finally I will discuss the pros and cons of each described method.

TU 206

Evaluation of available frameworks for mixture risk assessment in biocide and plant protection products in the EU
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Pesticides are regulated in the EU by the Biocide Product Directive (91/414/EEC) (BPD) and the Plant Protection Product Regulation (Regulation (EC) No 1107/2009) (PPR Reg). A new Biocide Regulation (BP Reg) is proposed and will likely come into force during 2014. Environmental and human health risks are evaluated before a product may be authorised and sold on the European market. Exposure to several substances based on the use of biocidal products and plant protection products are to be evaluated, according the PPR Reg and the BP Reg, respectively. This is more explicitly stated in the BP Reg compared to the BPD. Guidance on how to perform mixture risk assessment on biocide or plant protection products is very limited. However, several frameworks for tiered risk assessment of the combined effect of substances within products have been suggested or are being developed by e.g. WHO-IPCS and Competent Authorities from several EU Member States, such as DE, DK, ES and SE.

In the present work several such frameworks have been evaluated for risk assessment of both environmental and human health. We have used model products which are based on real biocidal or plant protection products, and the outcomes of the evaluations are compared from a regulatory decision making perspective. The suggested frameworks are all based on tiered approaches that include various levels of precaution and conservatism in order to be sufficiently protective of human health and of the environment.
even in data poor situations. The evaluation of the frameworks provides estimates of how protective and conservative they are in realistic regulatory assessments for both human health and environmental risk assessment.

The various frameworks and decision trees are designed to help with risk assessment on higher biological organization, i.e. easier to apply to environmental risk assessment than to human health risk assessment. Suggestions on how to prioritize refinement efforts in the product datasets should be included in forthcoming guidance.

TU 207

**DPD+: an appropriate method to assess the risk for mixtures under REACH?**

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According to REACH chemical substances need to be registered at the European Chemical Agency in Helsinki. For hazardous substance produced in amounts above 10 t/y the manufacturer or importer of the substance has to conduct a risk assessment and document the assessment in a chemical safety report (CSR). Operational conditions (OC) and individual use scenarios (IUS) for the substances have to be considered in the CSR. If deviations or reactions of the manufacturer or importer have to create a safety data sheet (SDS) for substances to communicate chemical safety of use within the supply chain. ES are attached to the SDS. The formulators must assess the risk for the mixture they produce and create a SDS for the mixture on the basis of the information they get with the SDS and ES for the single substances.

Although emissions to the environment results mainly from mixtures there is no guidance to assess the risk resulting from the use of mixtures. Industry developed the DPD+ method (based on the dangerous preparation directive) to assess the risk from mixtures. Aim of the method is to consolidate the information in the SDS and ES of the single substances to derive criteria of safe conditions of use for the mixture. The method is derived on the identification of a lead substance based on the classification of the substances in the harmonized classification, which is not considered in the derivation of OC and RMM for the mixture. The Federal Environment Agency, Germany (UBA) conducted a study to analyse the DPD+ method. The analysis tried to identify which potential risks of a substance could be overlooked by DPD+ and would hence not be adequately addressed in the selection of lead substances and the derivation of safe conditions of use. The above considerations question if DPD+ is a reasonable instrument to be used in the environmental risk assessment of mixtures (of herbicide, pesticides, pharmaceuticals, etc.). Whether or not the method has to be further elaborated and included in a method to assess the risk of mixtures. The interpretation of DPD+ (only substances with classified for environmental hazards need to be addressed in the risk assessment for the environment) is regarded as not in line with the interpretation of the legal text by Commission, ECHA and MS. Beyond that the possibility of an integration of scientific methods for the assessment of mixtures in the regulatory framework should be investigated.

TU 208

**Application of the TTC Concept to focus cumulative exposure assessment in environmental media: a special case for pesticides?**

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The concept of Threshold of toxicological concern (TTC) is based on establishing an exposure level for chemicals below which no significant risk is to be expected. This level is used to set a much lower exposure level for chemicals for which no adverse effects have been seen in traditional modes of toxicological testing. Especially for mixtures, the necessity to have data for every single chemical to make a risk assessment is of great disadvantage.

An overview of TTC methods is given, drawbacks of TTC based on structure and complaints to rule out the applicability of TTC to assess the risk of mixtures.

TU 209

**Relevant potency threshold: reducing uncertainty by calibration of cumulative risk assessment**

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Relative approaches, i.e., toxic equivalent (TEQ)-like approaches, for mixtures risk assessment have been established for some time. TEQ-like approaches assume that if certain premises are met, e.g., chemicals are true congeners, are metabolized and detoxified by the same biological processes, produce the same spectrum of biological effects by the same mode of action, and exhibit parallel dose response curves for the biological effects being measured (Safe 1990), then those chemicals may be assumed to be dose addative (DA) for specific toxicological effects. A recent report entitled “Potency and Sensitivity Testing for Hazardous Substances: Application to food ingredients” was published by the National Research Council (NRC) in 2006. The report recommended that the develop TEQ method should be conducted to facilitate the consolidated of environmental information on mixtures. The report recommended that the potential use of the TEQ method should be investigated.

TU 210

**Application of delayed fluorescence to estimate influence of chemical mixtures on alga**

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Algae are important primary producers in aquatic ecosystems. The OECD Test Guidelines 201 (TG201), algal growth inhibition test, is a conventional method that evaluates chemical mixture toxicity. However, conventional methods are not efficient in the evaluation of chemical mixture toxicity. In addition, the method (based on the dangerous preparation directive) to assess the risk from mixtures. Aim of the method is to consolidate the information in the SDS and ES of the single substances to derive criteria of safe conditions of use for the mixture. The method is derived on the identification of a lead substance based on the classification of the substances in the harmonized classification, which is not considered in the derivation of OC and RMM for the mixture. The Federal Environment Agency, Germany (UBA) conducted a study to analyse the DPD+ method. The analysis tried to identify which potential risks of a substance could be overlooked by DPD+ and would hence not be adequately addressed in the selection of lead substances and the derivation of safe conditions of use. The above considerations question if DPD+ is a reasonable instrument to be used in the environmental risk assessment of mixtures (of herbicide, pesticides, pharmaceuticals, etc.). Whether or not the method has to be further elaborated and included in a method to assess the risk of mixtures. The interpretation of DPD+ (only substances with classified for environmental hazards need to be addressed in the risk assessment for the environment) is regarded as not in line with the interpretation of the legal text by Commission, ECHA and MS. Beyond that the possibility of an integration of scientific methods for the assessment of mixtures in the regulatory framework should be investigated.

TU 211

**Species sensitivity distribution for the prediction of herbicides mixtures toxicity on benthic diatoms**

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Coastal zones of lakes could be contaminated by a cocktail of substances coming from urban discharges or diffuse watershed run off. This type of contamination especially concerns micro pollutants such as pesticides. Benthic diatoms, which are a major component of benthic biomass, are then exposed to several pesticides that could interact together, which might lead to unexpected results. Species Sensitivity Distribution (SSD) models are partly used to extrapolate protective concentrations for a community exposed to a single or a cocktail of contaminants. To predict the toxicity of a mixture, two concepts are used depending on the mode of action of the mixture components (Concentration Addition (CA), or Independent Action (IA)). The main objective of the study was to assess if SSD (using CA or IA models) were reliable in terms of toxicity prediction of herbicide mixture on benthic diatoms and if this SSD could be used as a management tool for aquatic ecosystems. As a first step, eleven species of benthic diatoms were exposed to 4 herbicides, separately (diuron, isoproturon, terbutryn, atrazine), in 96h monospecific growth inhibition tests.

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Finally, we compared the toxicity thresholds predicted by CA and IA models to the measured toxicity thresholds. We also assessed the species ranking between the mono herbicide SSD curves and the mixture SSD curves.

TU 212
Effects of Atrazine and 2,4-D mixtures on Lemma minor
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Thermal power sector is very important in terms of the economy. Pesticides are therefore widely used in Thailand and this usage is increasing. As a wide range of pesticides is used in Thailand, aquatic life will be exposed to a mixture of compounds. In order to understand pesticide impacts, it is therefore important to understand the potential toxic interactions of pesticides in use in Thailand. The aim of the present study was therefore to test the interactive effects of two herbicides, atrazine and 2,4-D, on Lemma minor. The study was carried out in the laboratory and in the field.

The effects of atrazine and 2,4-D on their own and in mixtures were assessed using OECD method 221. For the single compound studies, the effects of a range of concentrations of pesticide on the growth of L. minor were studied. For atrazine the test concentrations were 0.05, 0.1, 0.2, 0.4 and 0.8 mg-L⁻¹ and 2,4-D were 5, 10, 20, 40, and 100 mg-L⁻¹. The test was carried out on Lemma minor in 125 mL of nutrient solution.

Results from the single substance studies showed that atrazine was more toxic to L. minor than 2,4-D. Due to 2,4-D being an auxin hormone, it is absorbed through the leaves and translocated to the meristems of the plant in dicots and it does not affect monocots which may explain the low toxicity to L. minor. The studies into the combination effects of atrazine and 2,4-D on L. minor clearly showed that the two compounds interact in an antagonistic manner. The antagonism has been shown to occur more frequently in mixture where the herbicide mixture belongs to different chemical groups and monocot species.

In terms of this mixture study, the results showed that the interaction between atrazine and 2,4-D was antagonistic. These results are re-assuring and indicate in terms of impacts on aquatic macrophytes in Thailand, mixture effects are less than additive. If additivity is assumed for risk assessment purposes for macrophytes in Thailand, then this should be protective. Work is ongoing using a wider range of pesticides and test organisms.

TU 213
Predictive mixture toxicity assessment of pesticides in Swedish surface waters
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It has been demonstrated that combined effects of chemical mixtures give rise to stronger toxic effects than any of the compounds applied individually. The Scientific Committee on Health and Environmental Risks (SCHEER) has recently put forth concentration addition as a conceptual basis for the determination of environmental quality standards for mixtures. In this study we have predicted the risks from pesticide mixtures within Swedish surface waters using the concentration addition concept. Data from the national pesticide monitoring program in Sweden has been combined with experimental data from the US EPA’s database for single chemical toxicity and predicted toxicity to QSAR’s to determine the risks from pesticide mixtures in Swedish surface waters close to agricultural areas. The Swedish monitoring program has been taking samples in six different locations since 2002 and currently analyses the samples for more than 80 compounds, out of which 78 has been detected. All and all 751 weekly samples have been analysed bringing the total number of analyses performed close to 60 000. The abundance of information enabled the study of not only the individual site risks but also regional trends. Among the chemicals tested, average false rate is a problem when ignoring mixture effects as well as the sensitivity of the risk assessment to different ecotoxicological data has been determined. The results show that the average false positive results of the mixtures from the different sites range between 2.3 and 18.9, thus calling for additional assessments. The study also show no effect on risk from season implying that potential for recovery from pesticide stress is very limited. Finally the high ratio between predicted risk of the mixture and the average risk of the individual substances clearly demonstrates the need for going beyond the standard compound-by-compound assessment.

TU 214
A proposal for considering mixture toxicity with EQS compliance checking
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Environmental quality standards (EQS) are used under the EU Water Framework Directive to determine the chemical status of a water body. At present, compliance with EQS is only checked on the single substance level. This is based on the lack of evidence from mixture toxicity studies indicates that this procedure might underestimate the toxicity of the chemicals present in a water body, as the effect of mixtures has been shown to be higher than the highest single substance effect in the majority of cases. This propensity to underestimate potential effects of mixtures is underlined by a recent review published by Kortenkamp and co-workers. Two approaches have been proposed to date to account for mixture toxicity in assessing the chemical status: (i) application of a default assessment factor of 100 as used for EQS derivation by RIVM (NL) and (ii) application of mixture toxicity models based on species sensitivity distributions (SSD). The latter method predicts the ‘multi-substance potentially affected fraction’ (msPAF) of species proposed by Posthumus and co-workers. In this paper we propose a third approach for cases where the EQS was not derived from SSDs for all mixture components, which allows for more accurate assessment of environmental mixtures even where there is incomplete SSD information. The approach is based on the identification of a single representative taxonomic group and can be combined with the mixture cumulative ratio (MCR) approach recently proposed by Price and Han. It will also take into account the fact that specifically active substances will only be specifically toxic to some taxonomic groups while exerting baseline toxicity to other taxonomic groups. This novel approach will be illustrated using case studies based on real monitoring data and published EQS values. The everyday applicability of the approach will be discussed along with the decrease in uncertainty that it achieves when assessing the chemical status of water bodies containing more than one substance of concern.

TU 215
Increasing the number of data points does not necessarily reduce the probability of erroneously conclusions about interactive effects in mixture toxicity experiments
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The number of data points in mixture ecotoxicity studies is increasing exponentially. Developing requirements in regulatory risk assessment are further fueling the need to gain more knowledge about mixtures, mixture experiments and the subsequent data analysis. In particular, little attention has been given to the choice of an appropriate experimental design for a given reference model (e.g. independent action and concentration addition). Indeed, it has been shown that some experimental designs might not be able to detect (false negative) or erroneously conclude (false positive) deviations from a reference model. In this study, we simulated experimental data from a full factorial and equitox design and subsequently tested if these data deviated significantly from the concentration addition model reference experimental data. Experimental models were simulated for synergistic, antagonistic and additive mixtures. We then determined false positive and false negative rates for each experimental design. The number of data points, the lowest concentration of each chemical and the step size between two subsequent concentrations were varied. Results demonstrated a marginal increase in false positive rates with increasing number of design points. This contrasts with the general belief that adding design points will reduce the false positive rate. Varying the two other parameters resulted in a significant decrease in false positive rates. An optimal combination of these two parameters leads to the lowest false positive rates for a given design. In conclusion, we propose that the most appropriate experimental design for mixture toxicity studies is one that optimizes the lowest tested concentration and the concentration step size rather than increasing the number of design points. Furthermore, datasets based on suboptimal or imperfect designs lead to erroneous conclusions and care should be taken in analyzing and evaluating such datasets.

TU 216
Proposed PAH relative potency factors will greatly increase risks at all sites with PAH mixtures
B.H. Magee, D.W. Chin
TU 217
Derivation of environmental risk limits for polycyclic aromatic hydrocarbons (PAHs) based on internal residues
E.M.J. Verbruggen

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Polycyclic aromatic hydrocarbons (PAHs) are substances that have both natural and anthropogenic origins. They can be formed as a result of combustion, and are constituents of many petroleum products as well. Environmental risk limits (ERLs) were derived for 16 PAHs that were also considered in the European Risk Assessment Report (EurRAC) and are used for the evaluation of PAH contamination in suspended and particulate matter.

It can be assumed that toxicity of all PAHs is similar and possibly caused by carcinogeticness. The toxicity of different PAHs differs only as a consequence of different environmental distribution and accumulation potential. The sum of the internal concentrations of different compounds gives rise to the same effect as that of a similar concentration of an individual compound. This is referred to as concentration additivity. This concept has been successfully applied before for total petroleum hydrocarbons (TPH) and will be investigated here for PAHs.

All ecotoxicity data were collected and carefully evaluated for their usefulness and reliability. To calculate the total internal residues, pore water concentrations were calculated in each compartment, by considering partitioning between organic carbon and water. From water concentrations, the internal residues were calculated using a partition coefficient between the membrane and water.

From all chronic toxicity data collected and reviewed, a set of no observed effect residues (NOERs) for 54 species was obtained that showed no significant effects on biological parameters (e.g., biomass, growth) and between the individual PAHs, which yields the assumption that indeed accumulation from (pore) water is the determining factor for toxicity. On basis of these data, a species sensitivity distribution (SSD) was constructed, which appeared to be very similar to the SSD for TPH, suggesting a similar mode of toxic action. To derive a value for a generic ERL, an assessment factor of 5 has been applied to the HCS to account for the potential of certain PAHs to exert a high acute toxicity through photo toxicity.

For the PAHs it is confirmed that the equilibrium partitioning method is a useful method in setting quality standards. Because toxicity is driven by equilibrium partitioning, monitoring of these PAHs could be focused on measuring free water concentrations, e.g., in pore water, with phase extraction techniques.

TU 218  
Prediction of the mixture toxicity of PAHs and their derivatives in the Ah receptor based HHIE-luc assay

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Polycyclic aromatic hydrocarbons (PAHs) form a large group of widespread pollutants of high environmental concern. Many of the PAHs are toxic and exposure can result in mutagenesis and carcinogenesis in humans and animals. PAHs are transformed to oxygenated PAHs by chemical reactions in the air and metabolic reactions in organisms. Oxygenated PAHs (oxy-PAHs) are mutagenic and in addition more mobile than their parent PAHs. Today, the generic guideline of PAH contaminated areas are based on the 16 U.S. Environmental Protection Agency (EPA) priority PAHs, even though often hundreds of PAHs and PAH derivatives exist in the soils. Aaryl hydrocarbon receptor (AhR) activation is a central key event in the toxicity of many PAHs, which regulates the expression of cytochrome P450. PAHs are in general able to coactivate multiple transcription factors, and indeed accumulation from (pore) water is the determining factor for toxicity. On basis of these data, a species sensitivity distribution (SSD) was constructed, which appeared to be very similar to the SSD for TPH, suggesting a similar mode of toxic action. To derive a value for a generic ERL, an assessment factor of 5 has been applied to the HCS to account for the potential of certain PAHs to exert a high acute toxicity through phototoxicity.

For the PAHs it is confirmed that the equilibrium partitioning method is a useful method in setting quality standards. Because toxicity is driven by equilibrium partitioning, monitoring of these PAHs could be focused on measuring free water concentrations, e.g., in pore water, with phase extraction techniques.
set 223 Prediction of mixture toxicity for metals in soil: a reality check
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Euroumetexs, Brussels, Belgium
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Today, chemical pollution levels in soils are based predominantly on assessments carried out on individual substances. However, mixture toxicity is an upcoming issue in the regulatory area because of growing concern about the potential adverse effects of interactions between substances when present simultaneously. Only a few models are available so far to predict mixture toxicity (dose/concentration addition and independent action), and dose/concentration addition seems to be commonly selected as a default for the interaction prediction of higher order of mode of action (MOA) of mixtures. The applicability of these models in a regulatory framework for assessing the ecological effects of naturally occurring elements, as well as metals, in soil may not be straightforward, however. For several metals, the predicted no-effect concentrations are close to their natural background concentration in soil, and adding the potential effect of several metals may lead to over-protective regulation by setting the no-effect concentrations for mixtures in uncontaminated natural soils. Consequently, a reality-check is carried out on the use of common models for assessing the mixture toxicity of metals in soils and the selection of the number of metals to be included, based on exposure data for several metals in arable land and grassland soils across Europe (data from the GEMAS project). The distribution of the predicted total risk ratio across arable land or grassland in Europe is evaluated with respect to the mixture model applied, the number of metals included, the incorporation of bioavailability corrections, etc.

224 Ecotoxicological evaluation of chemical mixtures: posing hazard to human and environmental health
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Exposure to environmental chemical mixtures found in the environment can occur in different forms, with regard to reason of the contact and composition of the chemical mixture. Beyond all doubts is the fact that the chemical risk assessment, due to higher and more generalized contamination of environment with multi-component chemical mixtures, plays crucial role in toxicology and ecotoxicology. This work gives the most important issues concerning toxicology of chemical mixtures, with reference to an experimental design and special attention paid to the types of study methods, mainly acute and chronic studies, as well as legal aspects relating to European regulations connected with REACH and CLP.

225 Assessing human health risk from farmed milkfish consumption: considering toxic interaction among arsenic, copper, and zinc mixture
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Milkfish has been identified as a preferred fish species for farmed aquaculture in many countries worldwide. According to the Food and Agriculture Organization of the United Nations, milkfish (Chanos chanos) in farmed milkfish (Chanos chanos) consumption in blackfoot disease (BFD)-endemic area in Taiwan. The interaction risk assessment model proposed by the U.S. Environmental Protection Agency (EPA) and Agency for Toxic Substances and Diseases Registry (ATSDR) was used to estimate the interaction-based hazard index (HIINT) of non-carcinogenic mixture toxicity. The mixture toxic interactions of As/Cu and As/Zn were both antagonistic, whereas Cu/Zn was additive, with a maximum potential of about 70% of the HIINT of each substance. The application of these models in a regulatory framework for assessing the potential threat to human health posed by the mixture toxicity of metals from seafood consumption provide an illustration for assessing the potential threat to human health posed by the mixture toxicity of metals from seafood consumption.

226 Whether BDE209 interacts with Cd hepatotoxicity?
M. Canedi1, S.A.N. Stankovic1, S.A.A. Jankovic1, V.E.S.N.A Jacevic1, S.A.L.A.Vicunic1, K. Durgo1, B. Antonijevic1
1University of Belgrade, Faculty of Pharmacy, Belgrade, Serbia
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3Institute of Meat Hygiene and Technology, Belgrade, Serbia
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During the last ten years, mixture toxicology has undergone a remarkable and productive development. In fact, combined toxicity, which more accurately represents real environmental conditions, may actually assist in the determination of toxicologically relevant effects. The aim of this study was to examine whether decabromodiphenyl ether (BDE 209) influences cadmium (Cd) hepatotoxicity. Wistar rats (200-240 g) were exposed orally to BDE 209 (1000 mg/kg/bw), Cd (2.5, 7.5 or 15 mg/kg/bw) or their three combinations, by gavage, for 28 days. Control groups were exposed to saline or DMSO as a vehicle. The following end points were examined: liver weight, morphology, histology as well as blood enzyme activities (aspartate aminotransferase-AST, alanine aminotransferase-ALT, and γ-glutamyltransferase-γ-GT). Study was approved by Ethical Committee of Military Medical Academy (No. 9687/11). Liver weight increased in all groups comparing to controls. Interestingly, the highest increase was seen in Cd groups (20-42%), than in BDE209 group (28%), while in mixture groups it ranges between 96 and 115%. Morphological changes in liver were not observed while histological analysis indicated degenerative changes in hepatocytes, bile ductules, vascular bleeding and disruption of vascular membranes. Average histological scores on scale from 0 to 5 were: 0.13, 1.26, 2.26, 3, 3.4, 2.03 and 2.26, for control, BDE2091000, BDE2091000+Cd2.5, BDE2091000+Cd7.5, BDE2091000+Cd15, Cd2.5, Cd7.5, and Cd15, respectively. Our study indicates that As, Cu, and Zn mixture exposure may pose potential non-carcinogenic risk to human consuming farmed milkfish. The present interaction risk assessment model provides an illustration for assessing the potential threat to human health posed by the mixture toxicity of metals from seafood consumption.

227 Waste recovery - a special treatment under REACH
P. Vermuelen, A. Vassart, P. Anthoine, N.M. Debeleccky
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According to a manufacturing process is considered any production or extraction of substances in the natural state. This includes chemical modification as well as mechanical processes. Although the entry products at recovery companies are mainly waste covered (by the Waste Regulation), the status of the end products might "escape to be waste", and thus fall under the REACH regulation, and thereby having a major impact on the legal obligations of the recovery operator. However, under certain conditions recovery operators can benefit from recovery exemption by applying Article 2.7(d) of the REACH regulation.

As the end products at recovery plants are often complex mixtures containing substances whose presence is not always intended, the process of substance identification is challenging. This is the case for the mixture of substances that is covered as heterogeneous, under the re-introduction of former waste products on the market, because this contributes to the recycling of natural resources and the products may have economic benefits in the time of rising commodity prices. Nevertheless, sufficient efforts should be made to assure safe use of these, potentially heavily classified, mixtures. In addition, the risk assessment should be performed in such a way that overestimation due to the application of conservative assumptions and worst-case scenarios is avoided. Even if this concerns mainly address issues related to metal-based waste products, some may also apply to other types of recycling industries.

228 Toxicity evaluation of disperse red 1 textile dye using freshwater organisms from different trophic levels - A PNEC proposal
Military Medical Academy, Belgrade, Serbia
Institute of Meat Hygiene and Technology, Belgrade, Serbia
Military Medical Academy, Belgrade, Serbia
University of Belgrade, Faculty of Pharmacy, Belgrade, Serbia
University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia
Toxicity evaluation of chemical mixtures poses a significant challenge, especially when dealing with complex products such as dyes, which are mixtures of a main dye, surfactants and other synthesis impurities. The commercial dye Disperse Red 1 containing 60% of the main dye N-Ethyl-N-(2-hydroxyethyl)-4-(4-nitrophenylazo) aniline of 0.6 gph, considering that this compound would be responsible for the toxicity of the commercial product. This value was based on the lowest NOEC 0.1 ppm (chronic activity P. subcapitata and C. dubia) divided by an assessment factor of 10 because no chronic assay for fish is available and additional 10 because of its mutagenic potential. More studies are needed to verify the mutagenic effect of this product in microcrustacea as well as monitoring studies in river waters that receive textile discharges to determine exposure concentrations.
Enzymes can with regard to their ecotoxicological profile be grouped in proteolytic and non-proteolytic enzymes. Within these two groups read-across can be applied.

Assessment used by a policy maker can make a difference to the quality of life and health of (animal or human) populations living at a certain location. In the context of the current Life Cycle Impact Assessment, human health impacts due to water consumption have only been addressed by two published methods so far. Both methods assess development. In this study, different types of uncertainty for water consumption impacts are analyzed and combined on the endpoint level, showing how uncertainty leading to additional uncertainty due to aggregation of spatial explicit characterization factors (CF) on regional or global levels. This lack is a main challenge for practitioners. Information can be added to spatially explicit CFs.

Industrial enzymes - an example of the environmental risk assessment of an UVCB (unknown or variable composition, complex reaction products or biological materials)

Based on the current knowledge on industrial enzymes and their intrinsic properties future ecotoxicological testing of enzymes may be avoided by applying read-across and data waiving approach.

In conclusion, environmental risk assessment of UVCB’s like enzymes requires an alternative approach compared to the risk assessment of ‘classical’ chemicals or well defined mixtures due to the biological origin.

A partial least squares based integrated addition model for estimating mixture toxicity J.W. Kim1, S.H. Kim1, G.E. Schumann2

Korea Institute of Science and Technology Europe, Saarbruecken, Germany

In this study, the virtual impossibility of accurate quantitative modelling to predict the outcome of natural processes on the Earth’s surface, due to the inability of environmental model makers to consider all possible sources of uncertainties in their models or due to their unrealistic or even false assumptions.

Pilkey and Pilkey-Jarvis [1] claim the “virtual impossibility of accurate quantitative modelling to predict the outcome of natural processes on the Earth’s surface”, due to the inability of environmental model makers to consider all possible sources of uncertainties in their models or due to their unrealistic or even false assumptions.

In Life cycle assessment (LCA) we do use models whose outcome is always affected by a certain level of variability and uncertainty. The numerical solution of these models is, in fact, a mathematical expression of the variability and uncertainty of the input parameters, respectively. However, living organisms and the environments are exposed to both types of chemicals at the same time and space. Therefore, the scientific perspective, it still needs to develop an integrated model to predict mixture toxicity from different chemicals practically, regardless of whether mixture components produce similar, dissimilar, or both similar and dissimilar modes of toxic actions.

The objectives of this study are to develop and evaluate a partial least squares-based integrated addition model (PLS-IAM) for not only to overcome the multicollinearity problem which can be occurred between the two independent variables, CA and IA, but also to combine them into the integrated addition model by using the latent variable.

In this study, the PLS-IAM was validated by different datasets on mixture toxicity. The results showed that the prediction capability of the PLS-IAM outperformed reference models, the CA, IA, and IAM based on ordinary least squares.

Using sensitivity analysis in developing a characterization model for noise impacts S. Cucurachi, R. Heijungs

CMC - Institute of Environmental Sciences Leiden University, Leiden, Nederland

Pilkey and Pilkey-Jarvis [1] claim the “virtual impossibility of accurate quantitative modelling to predict the outcome of natural processes on the Earth’s surface”, due to the inability of environmental model makers to consider all possible sources of uncertainties in their models or due to their unrealistic or even false assumptions.

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Quantifying and propagating uncertainty in regionalized impact assessment: the relevance of spatial aggregation J. W. Kim1, S. H. Kim1, G. E. Schumann2

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The majority of ecotoxicity tests required for REACH registration data waiving can be applied including long-term aquatic toxicity testing and toxicity to terrestrial organisms. These tests are considered not relevant due to the fast biodegradation of enzymes, the low absorption and bioaccumulation potential and because enzymes are primarily active in the soluble fraction. Thus only short-term aquatic toxicity tests are considered relevant for industrial enzymes.

Based on the current knowledge on industrial enzymes and their intrinsic properties future ecotoxicological testing of enzymes may be avoided by applying read-across and data waiving approach.

In conclusion, environmental risk assessment of UVCB’s like enzymes requires an alternative approach compared to the risk assessment of ‘classical’ chemicals or well defined mixtures due to the biological origin.
country level data might not considerably add to uncertainty. Such information helps to improve efficiency when dealing with regionalized LCA. Overall spatial aggregation added considerable uncertainties.

**TU 234**

Sensitivity analysis for archetypes development in impact assessment of chemicals

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Emission of chemicals is increasing over years and the related impacts are greatly influenced by spatial differentiation. Chemicals are usually emitted locally but, due to persistence and physical chemical properties, may exert global impact. Variability of environmental parameters may affect the fate and the exposure up to orders of magnitude of difference. Accounting for spatial differentiation of chemical impacts requires the use of multimedia models, at various levels of complexity (from simple box model to computational intense and high spatial resolution model). Trade-off between complexity of high detailed models and global applicability requires the development of suitable archetypes. In the present study, sensitivity analysis techniques have been applied to MAPPE (Multimedia assessment of pollutant pathways in the environment) to build archetypes of emissions and fate. MAPPE is an advanced, multimedia, spatially resolved (1x1 degree) model, that allows the evaluation of chemicals removal rates from air, water and soil. We evaluate the relative influence of substance properties and of environmental characteristics on the variability in the environmental fate. In particular, using a Monte Carlo framework applied a sensitivity based sensitivity analysis techniques to find out those environmental parameters explaining the higher share of the variability (namely the variance) in the model outputs. This information is crucial in order to define the environmental dimensions to be used for the definition of the archetypes of emissions and fate, accounting for major differences in the potential impact of the different pollutants. We present results of the analysis for the pesticide Lindane. The overall variability of the removal rate from air is up to 5 orders of magnitude, mainly driven by four out of the eleven environmental parameters proposed in the model. These four parameters were then used as basis for building archetypes.

**TU 235**

Dealing with uncertainties in UNEP SETAC toxicity model

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In the field of LCA, the issue of uncertainty generates a growing interest. Although this issue has not yet been systematically addressed in existing databases and models, it neither can be neglected, nor addressed too simply. Although uncertainty has partly been quantified in USEtox model, several questions remain open to date. A significant share of this uncertainty arises in model itself, based on the relationship between the data and the intended reality being modeled. As regular users of the USEtox model, we identify the following sources of uncertainties:

- substance for which no data is available, or missing input parameter value
- parameters for which only an inappropriate or inaccurate value is available
- data for which more than one value is available (multiple sources and conflicting information)

Given the relative complexity of the mathematical model, uncertainty propagation has been performed using a numerical method. Well known monte-carlo simulation has been applied to assess the variability of the input parameters (substance data and landscape model). This approach allows us to describe statistical properties of a characterization factor value, based on input data uncertainties. It can also be used to study parameters sensitivity.

Taking into account the fact that uncertainty in USEtox database cannot be fully captured within the input parameters variability, other approaches have been studied, which aims at reducing uncertainty.

In approaches to reduce the uncertainty, database documentation, substance coverage, as well as user interaction are key issues.

The determination of the true value for a parameter requires research, experiments and therefore time. Involving stakeholders, discussing and finally deciding on a consensus, so as the current model has been built, so the database should be.

Possibilities must be given to each user, to view, or even produce the database of a specific parameter. Reporting an error in a parameter value also have to be easy, as well as the procedure of validation of each parameter value.

Our practical experience and ideas, related to management of these uncertainties in USEtox model will be presented.

Those approaches have been implemented in a web application prototype that allows uncertainty calculation and documentation completion.

**TU 236**

Standard information modules as basis for LCA reliability in the manufacturing context

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In standard Information Multimedia modules approach is presented as methodology to reduce uncertainty in the Life Cycle Assessment. In particular such approach aims to common barriers in the modelling of complex systems within the manufacturing context. An application is illustrated with reference to the uncertainty characterization of environmental profiles referred to energy flows. The use of standard modules for environmental profile characterization seems to improve the comparison of different studies by polarizing the improvement analysis on key life cycle stages.

**TU 237**

Taking into account variance of primary data in decision making: a method for the building sector

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Life Cycle Assessment (LCA) can be a powerful tool to drive society toward sustainability if used for help to decision. Nevertheless decision step comes often early in the design process where very few data is available. To overcome this issue analysts are forced to use poor quality data or generic data even for foreground system description which is hurtful to results liability and could mislead the decision-maker. In this paper we focus particularly on construction sector which represents about 40% of the total energy consumed in Europe [1]. In the building field, help to decision comes at the planning phase. At this step, only rough data are available but still the planning manager has to choose the best environmental solution regarding its expectations among several building team proposals. The method presented in this paper allows decision-makers choosing between solutions taking into account variance of primary data. It is tailor made to building sector.

In the first step of the method, selection of key parameters is made taking into account both sensitivity of parameter on the results and variance of these parameters. Then standard deviation is propagated using tailor series. This method has been implemented in a real case where decision needed to be made between three building projects. The case study shows that the method presented in this paper is easy to implement and suitable for complex projects which are tough to describe completely. In some case, it is possible to identify the best building solution even with up to 50% standard deviation on some primary data.

**TU 238**

Uncertainty evaluation of LCA models input data using Monte Carlo Method

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This is a contribution to the development of methodologies in considering uncertainties in LCA studies. The main results are a solid methodology for dealing with uncertainties considering the stochastic LCA models that enable practitioners to validate their results. The case study consists of an acoustic panel made up of cotton fibers, polyethylene and scraps of jeans. Its LCA model is developed with SimaPro® software. The Monte Carlo tool available in this software is used to perform a Monte Carlo simulation. Another model, called Monte Carlo Inventory model, is developed in Matlab® codes, with the model inventory table and in the CML 2 baseline 2000 impact assessment tables, all exported from SimaPro®. The same steps are followed for both models. First, a sensitivity analysis is made in the determined LCA model. Next the random parameters are chosen and modeled as random variables associated to a probability density function. Next, the Monte Carlo Method is applied and the convergence is observed. Finally, the results are analyzed. Both approaches are compared in order to observe if the method based on the inventory has the same performance of the method based on the input data. The objective is to obtain a basis of comparison for the stochastic LCA model and the focus is on the methodology development.

**TU 239**

How certain can we be about LCA after 20 years of harmonisation and standardisation? A critical comparison of methodology and results from ten LCAs comparing disposable cups

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Harmonisation and standardisation of LCA-methodology started in the 1990ties. It happened more than once in this period that contradictory LCA-results were released for a same product (particularly packaging materials). This resulted in intensive scientific debate, and also heated societal debate, especially where product claims were involved. Differences in LCA-results at that time could often be traced back to different decisions and assumption related to product system boundaries, functional unit definitions, technology choices, data sets etc.

In the last two decades LCA-methodology development has made immense progress. We meanwhile have a solid LCA frameworks, ISO 14044 describes the procedures to be followed, and specifies the assumptions and decisions to be made in LCA. Comprehensive and detailed guidelines are provided by national handbooks that still show few differences, but have a greater overlap in methodology. With this in mind it interesting to evaluate whether the progress in harmonisation LCA-methodology has also resulted in harmonisation of LCA-results. For that purpose we evaluated 10 LCA studies, each comparing a range of different types of disposable cups for hot and cold drinks. We
Urban background levels of novel brominated flame retardants in ambient air in Southern Bavaria, Germany
S.H. Hadler, D.H. Huber, W.K. Korner
Bavarian Forest National Park, Augsburg, Germany
After the technical penta- and octabromodiphenyl ether mixtures (PBDE) were banned in the European Union in 2004 and deabrominated diethyl ether is not any longer permitted in electrical and electronic equipment since 2008, there is an increasing demand of ‘novel’ brominated flame retardants. At present, little is known about the concentrations of these substances in ambient air. Therefore, ambient air was collected between March and September 2011 at the urban background air monitoring station of the Bavarian Forest National Park in Eastern Bavaria. A total sample volume of 9600 - 19000 m³ was collected over a period of 35 days using cartridges with a glass fibre filter and two polyurethane foams. Two low volume samplers were run in parallel, 1,2,6-bis(2,4,6-trimethoxyphenyl)hexane (BTBPE), hexabromobenzene (HBB), pentabromothiophene (PTB), bis(2,4,6-trimethylphenyl) ether (TMBP), decabromodiphenyl ether (DBDPE) and were concentrated using solvent extraction (AE). The analytes were separated by fractionation on a sulfuric acid-silica column. Purification of the two fractions containing BTBPE and the other flame retardants, respectively, was done on a Florisil®-column and on basic alumina, respectively. After concentration, samples were analysed by GC-EI-MS. C- and d-brominated isomers were identified and quantified. The sampling site is located outside the city of Kelowna; the Chilkoot Trail (CT) crosses from the Alaskan Panhandle into northern British Columbia. MA, GM, FB and the ocean side of CT receive copious seasonal precipitation, while BG and the East side of CT are in rain shadows. PAH profiles were similar across soils, with nearly all analytes found in all soils. Principle component analysis was performed, and the results showed a significant contribution of soil type to contaminant levels. The highest total PCB concentration was found in GM soils, followed by FB, then MA and lowest in the soils of BG and CT. Thus, where precipitation was the same, proximity to sources appears to dictate the levels of PCBs. The seasonal variation of specific toxic effects of organic air pollutants from year-long sampling campaign
CETOCOEN (CZ.1.05/2.1.00/01.0001).

Seasonal variation of specific toxic effects of organic air pollutants from year-long sampling campaign
E. Möller1, Z. Xie1, M. Carlucci, R. Sturmi1, J. He2, R. Ehninghaus3
1Research Centre for Toxic Compounds in the Environment (RECETOX), Brno, Czech Republic
2Dep't Biomed. Veterans. Sciences and Toxicol. Centre, University of Saskatchewan, Saskatchewan, Canada
3Air pollution of the 4 described to produce many toxic effects including endocrine disruption. Evaluation of such types of effects cannot be based only on data from chemical analyses. That shows the importance of incorporating specific bioassays into evaluation part of the air pollution monitoring programs. Utility of the methods in monitoring of atmospheric pollution levels has been shown previously. It is known that levels of pollutants in atmosphere are closely related to type of pollution source and a season period. In our study, we focused mainly on the latter factor. Air samples were collected for one year at a traffic-burdened urban site in Brno (Czech Republic) and a village site about 8 km from Brno city to address the influence of city agglomeration as an air pollution source on the surrounding area. The samples were analysed for a wide range of parameters and pollutants. In this work, we show the seasonal variation of aryl hydrocarbon receptor (AhR) mediated effects, anti-/estrogenicity and anti-androgenicity. These parameters were assessed using in vitro reporter gen assays based on mammalian cell lines. This research was supported by GACR P503/10/P249 and CETOCOEN (CZ.1.05/2.1.00/01.0001).

Profiles and cold trapping of polymeric aromatic hydrocarbons and polychlorinated biphenyls in biological Reserve of Atol das Rocas (Brazil) by persistent organic pollutants
E. Cai1, M. Cai2, R. Ebinghaus3, Y. D. Ying3, F. Wania1
1University of Toronto Scarborough, Toronto, Canada
2Environment Canada, Downsview, Canada
3Helmholtz-Zentrum Geesthacht, Geesthacht, Germany
PBDEs were detected in concentrations from 0.08 to 5.1 pg/m³ with BDE-47 and BDE-209 being the predominating congeners. Among the alternative BFRs, Onychoprion fuscatus species and 20.5-27.9 ng g⁻¹ for the O. fuscatus species with predominance of lighters congeners (tri-, tetra- and pentachlorinated groups). The p,p'-DDE occurred in 88% of the samples of O. fuscatus and in the two samples (100%) of A. stolidus with respective values ranging from <0.19-2.45 ng g⁻¹ and 0.30-3.15 ng g⁻¹. HCB was found in 68% of the samples of MA, GM, FB and the ocean side of CT with a mean value of 0.55 ng g⁻¹ and in the adult individual of A. stolidus in value of 0.65 ng g⁻¹. PBDE 47, at concentration of 4.70 ng g⁻¹, was collected over a period of 35 days from March to September 2011. Air pollutants were described to produce many toxic effects including endocrine disruption. Evaluation of such types of effect cannot be based only on data from chemical analyses. That shows the importance of incorporation of specific bioassays into evaluation part of the air pollution monitoring programs. Utility of the methods in monitoring of atmospheric pollution levels has been shown previously. It is known that levels of pollutants in atmosphere are closely related to type of pollution source and a season period. In our study, we focused mainly on the latter factor. Air samples were collected for one year at a traffic-burdened urban site in Brno (Czech Republic) and a village site about 8 km from Brno city to address the influence of city agglomeration as an air pollution source on the surrounding area. The samples were analysed for a wide range of parameters and pollutants. In this work, we show the seasonal variation of aryl hydrocarbon receptor (AhR) mediated effects, anti-/estrogenicity and anti-androgenicity. These parameters were assessed using in vitro reporter gen assays based on mammalian cell lines. This research was supported by GACR P503/10/P249 and CETOCOEN (CZ.1.05/2.1.00/01.0001).

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3. Results and discussion

4. Conclusion

The filter effect of canopy was observed along the Mont Mars and higher factors was related to low altitudinal sites and atmospheric particle. K.

Seasonality of concentrations levels (higher winter than summer values) as well as the gas-particle distribution (more compounds higher reactivity, they are a subject to long-range atmospheric transport (LRAT) and can pose significant health risks. As such, they are among the pollutants most commonly monitored in the atmosphere.

The average value of the total PAHs (ΣPAHs) concentrations of gaseous and particle phase was 1860 and 176.1 pg m$^{-2}$ for the forest, and 1230 and 123 pg m$^{-2}$ for the clearing. Seasonality, the PAH concentrations in the forest canopy effect of forest clearing (2-4). This study aimed to compare atmospheric concentration of PAHs under the forest canopy and nearby clearing the Lys Valley, Aosta, Italy, on the slopes of Mont Mars.

2. Materials and methods

Passive atmospheric sampling (PAS) was performed both in the forest and clearing sites at 700 m, 1000 m, 1400 m, 1800 m, and 2400 m (a.s.l) the Lys Valley, Aosta, Italy, on the slopes of Mont Mars. 2 high-volume air samplers were used to collect air samples at 1400m for 4 sampling periods.

3. Results and discussion

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Passive atmospheric sampling (PAS) was performed both in the forest and clearing sites at 700 m, 1000 m, 1400 m, 1800 m, and 2400 m (a.s.l) the Lys Valley, Aosta, Italy, on the slopes of Mont Mars. 2 high-volume air samplers were used to collect air samples at 1400m for 4 sampling periods.

3. Results and discussion

3.1. Concentrations

The average value of the total PAHs (ΣPAHs) concentrations of gaseous and particle phase was 1860 and 176.1 pg m$^{-2}$ for the forest, and 1230 and 123 pg m$^{-2}$ for the clearing. Seasonality, the PAH concentrations in the forest canopy effect of forest clearing (2-4). This study aimed to compare atmospheric concentration of PAHs under the forest canopy and nearby clearing the Lys Valley, Aosta, Italy, on the slopes of Mont Mars.

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This paper reports on seasonal and spatial variability of size-specific particle-gas partitioning behavior of selected groups of legacy (dioxins and furans, pesticides) and emerging (brominated and fluorinated) pollutants. Samples have been collected from the urban and rural sites in the Czech Republic for the period of one year (October 2009 - October 2010) using a high volume air sampler equipped with a six-stage (< 0.49 µm; 0.49-0.95; 0.95-1.5; 1.5-3.0; 3.0-7.2; 7.2-10 µm) cascade impactor collecting particulate phase and polyurethane foam (PUF) plugs collecting the gas phase.

TU 251
Potential input of organic pollutants to the Mar Menor lagoon: estimation of seasonal air concentrations using passive and active air samplers
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2Institute of Chemistry, Nanoscience, San Pedro del pinatar (murcia), Spain
A significant fraction of organic pollutants enters air directly and can be transported far from their sources associated to particulate material or dissolved. Pesticides are widely used for agricultural treatments and poly cyclic aromatic hydrocarbons are formed as consequence of urban, transport and industrial activities. Mar Menor lagoon is the leading point of drainages from a large agricultural area where a variety of pesticides are used. This lagoon is surrounded by several villages that have, especially in summer, an intense touristic activity. There is also a military airport close to the lagoon and this area is influenced by a very industrialized pole, which is located at 20 km south.

There are not previous data of pesticides and PAHs concentrations in air of in this area. In order to estimate the levels of organic pollutants in air and the potential atmospheric input in Mar Menor lagoon, passive samplers consisting of polyurethane foam (PUF) disks housed in chambers were deployed at six sites around the basin of this lagoon. Replicate at each point and calibration with a DIGITEL active sampler (at one point) equipped with a PUF module are used to improve the confidence of the determinations and to estimate the sampling rates for the different detected species.

PUF samples have been extracted with hexane using a pressurized liquid extractor. The extract was concentrated and was analyzed gas chromatography with mass spectrometry. The quantification limits were lower than 10 ng/mL for the majority of the considered analytes. This method has been validated for more than 80 organic pollutants and poly cyclic aromatic hydrocarbons) and up to 23 have been identified. PUF samples from active and passive samplers. The range of estimated air concentrations is very wide (10 pg/m3-2 ng/m3), and depends on the specific compound, as consequence of the proximity of sources and environmental variables.

Seasonal and spatial patterns have been found for different groups of organic pollutants associated to the main local sources in each case. The larger air levels of antracene, fluorescence and phenanthrene were detected in the proximity to the airport, while pesticides have a more homogeneous spatial pattern, with lower air concentrations by the sea side. Also a clear seasonal pattern has been detected in the case of some specific pesticides, such as chlorpyridphos, with higher concentrations in spring and autumn.

TU 252
Biomonitoring of poly cyclic aromatic hydrocarbons by pine needles - analytical alternatives and levels in Europe
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2University of Copenhagen, Copenhagen, Denmark
3IDAEA-CSIC, Barcelona, Spain
4Technical University of Crete, Chania, Greece
The monitoring of pollutants is a crucial step to assess their environmental exposure to organisms. In general, this task is performed in their own habitat (sediments, soil, water or air), but biomonitoring offers the possibility to estimate the multi-route uptake of contaminants. Given its worldwide presence, adequate characteristics and low availability costs, vegetation has been chosen as a valuable matrix since the 1960s, allowing as a passive sampler of a wide range of compounds (especially of atmospheric origin). However, in the case of the heavier PAHs, to be deposited as particles in the surface. PAHs are widespread carcinogenic and mutagenic contaminants with natural and anthropogenic sources mainly associated to combustion processes.

This paper reports on seasonal and spatial variability of size-specific particle-gas partitioning behavior of selected groups of legacy (dioxins and furans, pesticides) and emerging (brominated and fluorinated) pollutants. However the routine analysis of these compounds is still challenging because of the low concentration at which they occur (especially in their high volatility and because of their common occurrence in laboratory instrumentation, including gas chromatography columns, septa and sorbent phases).In this work, different analytical schemes for the analysis of CVMS and IVMS in wastewater and biota is presented. The methods are efficient to separate the investigated compounds from the water samples and liquid-extraction method for biota samples. The analytes were identified and quantified with gas chromatography-mass spectrometry (GC-MS/MS) using selected reaction monitoring. The analytes measured were found to be related to the analysis of wastewater and biota samples, offering excellent reproducibility, recoveries 63-113% and 40-112% and limits of detection 400 and 1600 pg/g in wastewater and biota samples; respectively.

Finally, the occurrence of siloxanes in more than 30 wastewater treatment plants in Catalonia (NE, Spain) was assessed, and the results confirmed the presence of the selected siloxanes at concentrations of ng/L in the final effluents. On the other hand, the analysis of biota samples using an extraction step by ultrasound-assisted extraction was also attempted, and the occurrence of siloxanes in biota samples was confirmed, at 0.2-2 ng/g in the final effluents. The parallel samples were collected with daily resolution over one month during November 2011. The sampling rate was 16 m3 d-1. The method was based on an existing method for decamethylcyclopentasiloxane (D5). Modifications were made to account for the strong sorption of hexamethylcyclotrisiloxane (D3). The method was shown to be applicable to the IVMS, whereby L3, L4, L5 and L6 were analysed using tetrisakis(trimethylsilyl) ester as the surrogate standard. The concentrations were of the order of 0.4 ng m-3 for L3, 0.04 ng m-3 for L6, and 0.02 ng m-3 for L4 and L5. The difference between the concentrations measured in the parallel samples was in almost all cases < 10% the influence of air mass loading on the temporal variability of the concentrations of the different analytes will be explored.
certain congeners have been banned completely and are currently on the list of the Stockholm Convention on Persistent Organic Pollutants (1,2).

Companies CIBA-Geigy (now Syngenta), Bayer and BASF and by the EU in the project MOST.

By unpaved roads at approximately 20% and then feed materials. Results indicate this approach could be used in a number of different agricultural emission characterization scenarios.

In this work, we applied both mechanisms in the analysis of the reaction of aerosol-borne terbuthylazine with OH radicals in our simulation glass-smog chamber. Home experiments and flow tube experiments (or combinations of both) are assumed to be different.

We note that the adsorption could be affected by gas-particle equilibria, and results from different experimental setups (e.g., Chamber experiments and flow tube experiments) are assumed to be different.

A Langmuir-Hinshelwood fit of atmospheric reactions of OH radicals with semivolatile, aerosol-borne compounds in chamber experiments

Results indicate this approach could be used in a number of different agricultural emission characterization scenarios.

A Langmuir-Hinshelwood fit of atmospheric reactions of OH radicals with semivolatile, aerosol-borne compounds in chamber experiments.

The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift fraction has an fraction of approximately 0.7, whereas volatilization is predicted to have an fraction of 0.9.
TU 262

Distribution of linear and cyclic volatile methyl siloxanes in indoor air samples and implications for human exposure


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Cyclic (D3, D4, D5, D6) and linear (L2-L5) volatile siloxanes are widely used in personal care products and cosmetics, as well as in industrial applications (biomedical products, surface treatment agents, plasticizers and construction materials). These compounds are of environmental concern due to their volatility, persistence and tendency to bioaccumulate.

The purpose of this study was to investigate their occurrence and distribution in indoor air environments, including domestic (i.e. bathrooms, living rooms, boy/girls rooms) and non domestic (offices, schools, etc.) environments, in two countries. An extensive indoor air sampling campaign was performed contemporarily in the UK and Italy, between May and August 2011. Indoor air samples (n=10) were considered on adsorption Tenax GR cartridges (60/80 mesh, Markes) using conventional portable air sampling pumps (i.e. GilAir3, Gilian- Sensidyne), operating at a flow of about 120 mL min^-1. Sampling cartridges were desorbed using the Automatic Thermal Desorber UNITY2 coupled to a GC with a specific GC/MS-system. Concentrations of total siloxanes ranged from 20.6 to 467 ug/m3 and from 55.7 to 424 ug/m3 in Italian and UK samples, respectively. Cyclic volatile methyl siloxanes (in particular D3 and D5) accounted for ~90% of total siloxanes found in both countries.

The highest siloxane concentrations were found in bathrooms; in particular, D5 represented the principal compound probably due to its dominant presence in most hair products, skin lotions, cosmetics, and cleaning agents. Personal lifestyles and everyday-life behaviours (e.g., selection of products, frequency of application, amount applied) had a determinant effect on the amount of total siloxanes found in both Italian and UK adult and living rooms. No significantly different concentrations were detected in indoor environments. The daily inhalation exposure (DIE) to cyclic and linear volatile methyl siloxanes was also evaluated.

TU 263

Source contribution of atmospheric PAHs at urban and industrial locations in the city of Novi Sad, Serbia


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Data on polycyclic aromatic hydrocarbons (PAHs) in ambient air accessed at urban and industrial locations in the city of Novi Sad, Serbia, have been analysed in order to determine emission sources and their contributions to the concentrations of particle-bound PAHs. Previous studies have shown that the major contributors of PAHs in urban areas are the emissions from vehicle exhaust, and emissions release from industrial processes like aluminium production, creosote and wood preservation, waste incineration, cement manufacture, petrochemical and related industries, commercial heat/power production etc. The sampling campaigns have been conducted at three sampling sites during the 14-day period. One site was situated near industrial area in the city of Novi Sad, the second site was located nearby the heavy traffic area, especially busy during the rush hour. The third site was residential district. Non-heating sampling period lasted from June 26th to July 9th 2008, while sampling of ambient air during the heating season was undertaken from January 22nd to February 4th 2009. 104 air samples were collected using a high volume air sampler TCR Terc0 B/49010/ECHO HVol with quartz fibre filters (QFVs). 16 US EPA polycyclic aromatic hydrocarbons were determined in all samples using a gas chromatograph Shimadzu GC-2014 with a flame ionization detector. The total average concentrations of PAHs ranged from 0.4 to 3.16 ng/m³ during the non-heating period and from 0.05 to 3.61 ng/m³ in the heating period. Various techniques, including diagnostic ratio (DIR) and principal component analysis (PCA), have been used in the data analysis. Diagnostic ratios indicated that vehicles, diesel and/or gasoline, industrial and combustion emissions were potential sources of PAHs in Novi Sad. Additionally, principal component analysis was used to constrain the potential sources. The results show that vehicular emissions are the most important source of particle-bound PAHs in Novi Sad, Serbia. Less dominant emission of PAHs comes from stationary combustion sources such as home heating during the winter and oil refinery processes during the whole year.

TU 264

Polydominated diphenyl ethers (PBDEs) and other flame retardants in adult Pipistrellus bretschi (Pipistrellus spp) from an urban area of NW Britain

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Bat (Pipistrellus spp) livers of 30 adult males (M) and 23 adult females (F) from an urban area of the NW of Britain, were analysed for 23 PBDE congeners (Tri to hex-BDEs) as well as for other flame retardant (pentabromophenols, pentabromophenylmethane, hexabromobenzene, hexabromocyclododecane (HBCD) and the two isomers of Decabromane (DBD)).

The dominant PBDE congener were BDEs 47, 99, 100, 153, 154 and 183 in both M and F, which are the same dominant congeners present in the penta technical mixtures.

However, bats have a lower proportion of PBDE 47 than the Penta congeners, suggesting degradation of this compound. Total BDEs were dominated by penta-BDEs (65% of total BDEs (with just over 20%) for both males and F.

The sum of the PBDEs analysed showed a wide range (10.4-8191 ng g^-1 wet weight (ww)) of concentrations, with a mean of 961 ng g^-1 ww. Males had on average twice the concentration of PBDEs than F (M: 1182 ng g^-1 ww, F: 673 ng g^-1 ww), although this difference was not statistically significant, possibly a result of the small sample size.

Penta-BDEs were not detected in any sample and pentabromophenylmethane and hexabromobenzene were only found in a few individuals. In our samples we only could detect the anti isomer of DP, with concentrations between 1.6-38.4 ng g^-1 ww. This may have been expected because this is the dominant isomer in the technical mixture. However, we only found DP in M, this may be the result of F offloading accumulated residues to the foetus and to juveniles via milk, as DP is highly lipophilic. The lower residues of PBDEs in F than M is likewise consistent with transfer of residues from mothers to offspring. In contrast however, HBCD (sum of the 3 diastereomeric pairs (6.176 ng g^-1 ww), and this suggests that there may be sex differences in exposure to or metabolism of this compound. In conclusion, this is the first study that we are aware of to report tissue concentrations of PBDEs and some of their emergent replacement compounds in bats from Europe.

TU 265

Atmospheric pollution due to methane seepage from surface rock layers

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Methane emission contributes to the growing background concentration of ozone, an air pollutant that impacts on air quality and, hence, human health. In particular, methane emissions from surface rock layers into the atmosphere is a problem in many parts of the world. The ground surface is the zone where the processes of weathering are very active. In the presence of gas-bearing rock layers (gas sources) located at a shallow depth below the ground surface, weathering-induced fractures in surface rocks can conduct gas from gas sources into the atmosphere. In the southern part of Donetsk city (Ukraine), weathering-induced fractures in very weak rock (sand shale) are intervention processes. The values of fluxes of gas flow through fractures and intensity of gas flow were measured in situ. Observations show that number of fractures per meter measured along traces of channels is between 34 and 69, and total fracture length of through channels per unit area ranges from 19.5 to 21.6 m/sq meter. It is established that fracture aperture width along trace of through channel can reach 7 mm, and value of the fracture aperture tends to decrease with increasing depth below the ground surface. In situ study shows that role of the system of weathering-induced fractures in the process of gas emission to the atmosphere is much more significant than the role of rock matrix. Indeed, gas flow from through channels is 10.4-19.8 times larger than gas emission from rock matrix, and weathering-induced fractures conduct large portions (91-95%) of gas emitted from ground surface. The gas flow from individual through channel ranges from 0.00069 to 0.0041 m³/sq meter. The study of impact of methane seepage on human health shows that there are cases of methane poisoning of people living on the ground floor. The frequency (F) of the methane poisonings was statistically analysed. As a result, it is established that frequency F is dependent on average value of gas volumetric flow rate from individual through channel (m³/sq meter), number of through-rock-conducting channels (k) located within a 20 m radius around each dwelling house. It is defined that value of F increases according to exponential, where a variable exponent is the gas flow (m³) multiplied by logarithm of the number (k) of gas-conducting channels.

TU 266

Environmental impact of implementing alternative fuels in a Spanish cement plant

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Cement industry produces around 5% of the world's anthropogenic CO2 emissions, either as direct (desulphuration of calcium carbonate - raw material)- or indirect (fuel combustion) contributions. In order to reduce these contributions to CO2 by 60 to 87%, new environmental policies promote the use of alternative fuels such as sewage sludge and refuse derived fuel, among others. Because a reduction of CO2 and costs (fossil fuel saves) may be expected, this activity has resulted to be very environmentally and economically beneficial. On the other hand, the population living closest to the cement plants are generally concerned about the possible effects on the environment and health especially derived from the use of alternative fuels. In some populations it is especially important, as there is a short distance between cement plant and homes. This short distance is sometimes due to poor (or inexistent) urban planning, illegal construction and environmentally and economically beneficial. In the city of Catalonia, Spain, the cement industry has started to use different kinds of alternative fuels for partial replacement of the energy, which traditionally comes from fossil fuels. This will not only reduce the amount of CO2 emitted but will not generate a negative impact on the environment and health of residents around the cement plant, various environmental monitoring campaigns have been conducted in the surroundings of facilities of different characteristics, in terms of used fuel (sewage sludge, refuse-derived fuel, etc.) or zone characteristics (urban, suburban or rural). In each campaign, several samples of environmental monitors (air herbage and soil) were collected before and after the alternative fuel implementation, and the content of metals (phytotoxic and biotoxic) was determined in situ. Observations show that no significant differences between campaigns regardless of the use of alternative fuels. In conclusion, the current results support and encourage the
option of using alternative fuels in cement plants working with the Best Available Technologies (BAT).

TU 267
Should the neighbourhood of cement plants be concerned when alternative fuels are used? J. Rovira 1, M. Mazi, M. Nadal 2, M. Schuhmacher 1, J. L. Domingo 2

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In recent years, co-combustion of alternative fuels in cement plants has become an increasing practice in many countries. The benefits associated to the replacement of fossil by-products (sludge or sewage sludge and RDF) are environmental and economically (e.g., reduction of CO2 emissions, reuse of by-products) evident. These benefits are more important taking into account that cement production is one of the main CO2 emitting manufacture industries, but also due to the important raising of fossil fuel prices. Furthermore, considering the notable consumption of fossil fuels by the cement industries, the European Union is encouraging the enhancement of the amount of alternative fuels in cement kilns.

In the present work, the result of a 3-year study (2008-2011) environmental monitoring campaign around a cement plant located in the metropolitan area of Barcelona (Catalonia, Spain) was presented. The aim of this study was to evaluate the potential changes in the environmental levels metals and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in the vicinity of a cement plant before and after using sewage sludge as alternative fuel. The concentrations of PCDD/Fs and metals were determined in different soil, herbage and air samples around this facility before and after the partial fuel substitution. The potential changes on the health risks for the population living around the facility were also assessed.

In general terms, no statistically significant differences in the pollutant values were found between the 2 scenarios (before and after the partial fuel replacement), with the exceptions of a few significant decreases in some metals. Besides, the carcinogenic and non-carcinogenic risks due to the exposure to metals and PCDD/Fs were below the national and international safety limits.

TU 268
Environmental impact of mechanical-biological treatment systems. Human health risks of chemical and microbiological pollution L. Vilavert, M. Nadal, M. Schuhmacher, J. L. Domingo

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Due to legislative, environmental, economic, and social limitations, the identification of sustainable disposal pathways for the management of municipal solid waste (MSW) is a very challenging task. The mechanical-biological treatment (MBT) approach presents many advantages in comparison to other waste management possibilities. However, adverse health effects related to this practice are not negligible, such as dust, aspergillosis fungi, and chemical pollutants, among others. Volatile organic compounds (VOCs), for example, are generated during waste composting and similar processes. Some of these contaminants can be the cause of a variety of infectious diseases, as well as allergies and toxic effects or generating hazardous properties.

In 2010, a program was initiated to monitor air levels of VOCs and microbiological pollutants near a MBT plant in Montcada i Reixac, (Barcelona, Catalonia, Spain). To investigate the temporal and seasonal trends of chemical and microbiological pollutants, four 6-monthly campaigns were performed. Air samples were collected at different directions and distances from the facility.

The levels of total bacteria ranged from 160 to 1054 cfu/m³ (colonies-forming units per cubic meter) with a high increase in the last survey (summer 2011) compared to the initial (winter 2010). In fact, the highest concentrations were detected in the winter campaigns. Fungi at 37°C were also important in the hot seasons with values of 716 and 106 cfu/m³ (summer 2010 and 2011, respectively) versus 60 and 61 cfu/m³ (winter 2010 and 2011, respectively). The concentration of the remaining microbiological agents (gram-negative bacteria and the more specific Aspergillus fumigatus) was rather low, with a lack of temporal/seasonal differences. On the other hand, the highest mean concentration of VOCs was found in the third campaign (45.7 μg/m³), with values between 4.55 and 120.9 μg/m³. For these chemical compounds, the greatest concentrations were found in two winter campaigns.

The current exposure to those chemicals, estimated from the environmental burdens, should not mean additional non-carcinogenic or carcinogenic health risks for the population living nearby. Finally, a fate and transport model was also executed to validate the acquisition of data from outdoors, as well as comparing modelled and experimental results.

TU 269
Environmental risk management in confined spaces. A study case of an archive like a model of indoor environment involved with biodeterioration A. Michelau, S. Manente, R. Ganzerla, G. Ravagnan

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2Progetto specifico indoor environments, such as those that preserve Cultural Heritage, cannot fail to take into account the interactions they may have on human health. The Historical Archives, where cellulolytic microfungi and bacteria find a comfortable environment for their development, are of particular interest in this sense. In fact, one of the major causes of paper degradation is the biodeterioration, which develops with specific environmental conditions. Therefore, it is necessary to conduct research linking aerobiology with environmental Archives, to understand the conservation state of the documents and, also, the possible risks for the Archive's operators and for people who work within these environments.

The study case involves the Contemporaneo Political History Archives sites in Ca' Tron, Treviso (Italy) in the Veneto countryside. To this end, a microbial sampling and microbiological analysis were performed. Air samples were collected at different directions and distances from the facility. The levels of total bacteria ranged from 160 to 1054 cfu/m³ (colonies-forming units per cubic meter) with a high increase in the last survey (summer 2011) compared to the initial (winter 2010). In fact, the highest concentrations were detected in the summer campaigns. Fungi at 37°C were also important in the hot seasons with values of 716 and 106 cfu/m³ (summer 2010 and 2011, respectively) versus 60 and 61 cfu/m³ (winter 2010 and 2011, respectively). The concentration of the remaining microbiological agents (gram-negative bacteria and the more specific Aspergillus fumigatus) was rather low, with a lack of temporal/seasonal differences. On the other hand, the highest mean concentration of VOCs was found in the third campaign (45.7 μg/m³), with values between 4.55 and 120.9 μg/m³. For these chemical compounds, the greatest concentrations were found in two winter campaigns. The current exposure to those chemicals, estimated from the environmental burdens, should not mean additional non-carcinogenic or carcinogenic health risks for the population living nearby. Finally, a fate and transport model was also executed to validate the acquisition of data from outdoors, as well as comparing modelled and experimental results.

TU 270
Persistent organic pollutants (POPs) in ambient air, human breast milk and edible fish - a potential health risk for the Ghanaian S.O. Ado Kumi, J.K. Klaonova, K.A. Asante 1

1EPA, Accra, Ghana

The Environmental Protection Agency (EPA), Accra, Ghana, works towards improving the quality of the environment and the wellbeing of the population through environmental management. This work is underpinned by national and international safety limits.

In general terms, no statistically significant differences in the pollutant values were found between the 2 scenarios (before and after the partial fuel replacement), with the exceptions of a few significant decreases in some metals. Besides, the carcinogenic and non-carcinogenic risks due to the exposure to metals and PCDD/Fs were below the national and international safety limits.

TU 271
Toxicity screening of fine particles by a novel microbial test battery I.R. Gutierrez, D. Dietrich, W. Ahlb 1

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Universität von Konstanz, Konstanz, Germany

TU-Hamburg/ Harburg/Inst. of Energy and Environmental Techniques, Harburg, Germany

Bacterial monitoring on two SO 2 standards having been extremely popular worldwide to the increasing costs of fossil fuels. Claimed as renewable energy source, wood has often been ignored as a source for fine particles causing toxic effects. Rapid reproducible and validated biotests for toxicological characterization of such particles exist neither for cells nor for micro-organisms. Thus there is a need for established macroscale toxicity screening of unknown combustion born particles. Here a test battery consisted of two ISO standardised bacterial contact assays and one yeast biotest was modified for the use with fine dust. Having different endpoints these tests provide a toxicological “fingerprint”. In order to account for bioavailability of contaminants adsorbed on surfaces of fine particles, such were used as a whole not as extracts. Particles were measured electronically from different furnaces in the flue gas channel. Suspended in water, vortextted for 1 min, and ultrasonicated for 15 min samples were applied in a concentration range of 0.001 - 1.0 mg/mL. Particle sizes (5-10 μm) in these suspensions were measured by dynamic light scattering. The control contained no particles, solely deionized water.

Cytotoxicity in the aerobic Arthrobacter globiformis contact assay was determined via dehydrogenase activity (DHA). Genotoxicity was determined using Umu-Test using a $K_{Ja}$-Test with heterologous plasmids. Some preliminary results suggest suitability of endpoints determined in the modified tests for risk assessment of fine particles.

TU 272
Airborne particle matter induced pro-inflammatory effects and oxidative stress in A549 cells S. Michael, M. Montag, W. Dott

SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
Clean air is a basic requirement for human health and well-being. An average daily inhalation of 20 m³ of air is characterized by an exposure to many different pollutants. Apart from the classic gaseous pollutants airborne particulates continue to pose a significant threat to human health worldwide.

The objective of this study was to compare the toxicological effects of different source-related particles in regard to their chemical composition. In this context we investigate airborne PM from different sites in the Region of Aachen. A549 cells were exposed to increasing PM concentrations followed by analyses of cell viability, pro-inflammatory and oxidative stress response.

The results showed a seasonal and location dependent variation of the PM concentration for both particle fractions. Chemical analysis of these particles indicated the presence of 21 elements, water-soluble ions and a multitude of different PAH. The major inorganic components are the crustal elements (Ca, K, Mg, Na) and the (transition) metals Al, Fe, Zn. In contrast to metals and PAH, the concentration of inorganic species is only approx. 50-70 % higher at the rural site, compared to the urban site. Water-soluble particles (up to 100 %) and organic species (up to 10 %) indicate a high degree of atmospheric aging.

The results concerning the A549 cell line showed that exposure to ambient PM induced a concentration-dependent decrease in cell viability and an increase in pro-inflammatory and oxidative stress markers. The samples of the urban traffic location, characterized by a high concentration of elemental/organic carbon and metals, induced the highest pro-inflammatory and oxidative activity. Due to the combination of chemical-analytical and toxicological methods a characterization of PM induced cause and effects are possible.

The results of the ambient PM samples in this study were analyzed using various sampling methods and emission source regions in the region of Aachen. Our results support other investigations regarding the importance of the chemical compositions and there PM induced toxicity in vitro.

The study is embedded in the project City2020+ which is part of the interdisciplinary Project House HumTec (Human Technology Center) at RWTH Aachen University funded by the Excellence Initiative of the German federal and state governments through the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG).

EC06P - Sorption and bioavailability in sustainable remediation of organic chemicals

Impact of geosorbents on bioavailability of polycyclic aromatic hydrocarbons to humans by oral uptake

Norwegian Institute for Water Research, Oslo, Norway

Institute of Hygiene and Environmental Medicine, Aachen, Germany

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TU 276

Simulating the bioavailability of mineral hydrocarbons for earthworms using different extraction methods

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In the context of assessing the habitat function of contaminated sites only the bioavailable contaminant fraction of relevance. Information on the habitat function, therefore, can be obtained in ecotoxicological tests performed with representative soil organisms. Depending on the test organism the duration of effect measurements varies between several weeks and several months. In order to obtain results focusing on bioavailability faster, chemical methods are being developed to predict the amount of contaminant that can be taken up by a specific organism. Suitable methods have to simulate the respective exposure pathways and represent the bioavailable fraction of the contaminant.

Extraction procedures for mineral hydrocarbons were regarded with respect to their suitability for simulating the bioavailable fraction for soil organisms. As effect parameter the reproduction activity according to OECD Test Guideline 222 was selected. The test was performed in eight sandy soils that had been contaminated with mineral oil for many years. The results obtained in the earthworm studies were consistent with the concentration of the mineral hydrocarbon contamination using different extraction procedures: (I) total content using exhaustive extraction (C10 - C40 fraction); (II) fraction of C10-C22 performing exhaustive extraction; (III) water based extraction - shaking procedure; (IV) water based extraction - column procedure.

The best relation between an increased concentration of contamination and a reduced habitat function was detected for the C10-C22 fraction of the HPCD-extraction. Relations with the C10-C40 fraction of the HPCD-extraction and with the C10-C22 fraction of the exhaustive extraction were less pronounced. No relation was observed for the total content of contamination using shaking and column procedures.

In conclusion we demonstrated that the quality control criteria of EPA Method 1668 can be met using a GCMS triple quadrupole system. Advantages for the laboratory are the reduction in cost of the equipment relative to high-resolution GC-MS systems, along with the ability to use the system for more than just specialized applications. In addition, a library of transitions for the PCBs and other compounds simplifies method development and verification, simplifying the adoption of GC-triple quadruple MS for this analysis.

TU 279

Analysis of pesticides in water samples based on the combination of solid phase extraction and solid phase microextraction coupled to gas chromatography mass detection

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The aim of this study was to compare the chemical and biological soil properties and to obtain information on the fate and effects of pesticides in the field and in the laboratory.

The soil samples were taken from a field trial with different treatments and without treatment. The treatments were divided into three groups: (1) a control group; (2) a group with an application of herbicides and pesticides; and (3) a group with an application of fungicides and pesticides. In the laboratory, the soil samples were divided into two groups: (1) a group with an application of herbicides and pesticides; and (2) a group with an application of fungicides and pesticides.

The results showed that the different treatments led to different effects on the biological soil properties. The biological soil properties were measured using the respiration activity test and the seedling growth test. The results showed that the treatments with an application of herbicides and pesticides led to a decrease in the respiration activity and a decrease in the seedling growth.

The results of the field trial showed that the treatments with an application of herbicides and pesticides led to a decrease in the respiration activity and a decrease in the seedling growth. The results also showed that the treatments with an application of fungicides and pesticides led to an increase in the respiration activity and an increase in the seedling growth.

The results of the laboratory trial showed that the treatments with an application of herbicides and pesticides led to a decrease in the respiration activity and a decrease in the seedling growth. The results also showed that the treatments with an application of fungicides and pesticides led to an increase in the respiration activity and an increase in the seedling growth.
Reduced (e.g., historical pollution), sediments can become a secondary source to their surrounding environment. As a result of the challenges encountered when attempting to predict contaminant bioavailability and mobility, gauging the risk posed by these contaminated sediments remains challenging.

In an effort to control and reduce the impact of contaminated sediment on ecosystems and human health, a risk assessment guideline tool for contaminated marine sediments was recently developed within the framework of the project "Contaminated Marine Sediments: Comparison of total sediment concentration (TSC) to 10%". In many real-life situations, the TSC-based risk assessment is used to determine which contaminated sediments need to be addressed. In this study, the comparison of TSC to 10% revealed that a large number of the studied sediments have a significant risk. This suggests that a more detailed risk assessment is necessary to identify the most contaminated sediments.

To address this problem, a novel bioavailability test was developed. The test is based on the concept of a sink, a site where pollutants are removed from the environment. The sink is used to determine the bioavailability of contaminants in sediments. The test involves the measurement of the amount of contaminants that can be released from the sediment under different conditions. This information is then used to predict the potential risk posed by contaminated sediments.

The test was applied to 25 Austrian soils, which were all collected from non-industrialized areas. The soils were spiked with four selected priority polycyclic aromatic hydrocarbons (PAHs): Phenanthrene, Fluoranthene, Benzo(a)pyrene and Chrysene. The soils were then exposed to two different media: artificial digestive fluids and laboratory simulated digestive fluids. The results showed that the bioavailability of PAHs in the soils was significantly higher in the artificial digestive fluids than in the laboratory simulated digestive fluids. This suggests that the artificial digestive fluids are more effective at removing PAHs from the sediments.

The study also showed that the bioavailability of PAHs is influenced by the contaminant matrix, the extent of sorption site coverage and the contamination's age. Moreover, the results indicate that the bioavailability of PAHs in sediments is not only affected by the sediment's properties but also by the environmental conditions.

In conclusion, the results of this study suggest that the novel bioavailability test is a promising tool for assessing the bioavailability of contaminants in sediments. The test can be used to identify the most contaminated sediments and to prioritize the remediation efforts.
efficiency in increasing biodegradation performance, and its dependence on environmental conditions, has hitherto not been conducted. Using a microbial simulation model, we therefore investigate bacterial degradation performance in response to networks that either act as bacterial dispersal vectors (‘highways’) or as contaminant translocation vectors (‘pipelines’) or as a combination of both. We analyse biodegradation improvements compared to the situation without networks, and systematically test a variety of spatially homogeneous and heterogeneous networks. We find that each mechanism can improve biodegradation performance. The degree of improvement, however, may vary distinctly depending on the environmental conditions, and may also be negligible under certain conditions. Particularly, networks acting as ‘highways’ allow bacteria to overcome motility restrictions and reach remote areas, whereas networks acting as ‘pipelines’ may initiate degradation by bringing remote contaminants to bacteria. As a consequence, highest biodegradation improvements often emerge from the combination of both mechanisms. We therefore conclude that ‘fungal highways’ as well as ‘fungal pipelines’ should be considered for developing novel bioremediation strategies based on fungus-mediated transport. Future experimental studies should focus on detection and appropriate stimulation of the two mechanisms in typical bacteria-fungi associations in contaminated soils.

TU 286

Influence of rhizosphere oomycete mycelia on bacterial biodegradation of phenanthrene present in nonaqueous-phase liquids (NAPLs)

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Two rhizosphere oomycete species (Pythium aphanidermatum and Pythium oligandrum) were tested for their influence on biodegradation of phenanthrene present in NAPLs by the soil bacterium Mycobacterium gilvum VM552. Both species showed no antagonistic effects on the bacterium in co-culture assays. An optimized production protocol was established for oomycete mycelia using a direct scrape-out method with solid agar, which prevented the contamination by carbon-rich medium components. Mineralization of 14C-phenanthrene present in hexadecane-fuel-NAPL mixtures by M. gilvum VM552 was increased in the presence of P. oligandrum mycelia. The fungus showed a hierarchic fission stage for mineralization and delayed the mineralization plateau. However, there was no stimulation of mineralization when the NAPL was composed of a heptamethylbenzene/fuel mixture. We suggest that fungal growth on biodegradable NAPL mixtures may promote the bacterial colonization of the NAPL/water interface, possibly by promoting cell adhesion and/or causing interface destabilization, thus increasing the bioavailability of PAHs for bacteria.

TU 287

Sunflowers in rhizoremediation: a possible alternative for improving the bioavailability of PAHs in soils

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The sunflowers are widely used in agriculture and in the environment as a bioavailability-promoting agent. In this study, we focus on the capacity of sunflower biomass to enhance the solubility of PAHs present in solid soils. We tested the effects of sunflowers on the solubility of PAHs and other organic compounds by incubating sunflowers in soil with different initial PAH concentrations. The results indicate that the establishment of an appropriate chemical activity gradient is critical for a positive or negative influence of HS on biodegradation.

TU 288

Biosurfactants and sustainable bioremediation: effects on slow desorption PAHs

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The use of biosurfactants is promising alternative for enhancing desorption of soil-sorbed PAHs and their bioavailability for microbial degradation. In this study, we demonstrated that biosurfactants can promote dissolution of soil PAHs and enhance desorption when they are present as rapidly desorbing fractions (> 0.1 h−1). However, not much is known about the effect of biosurfactants on bioavailability of slowly-desorbing hydrophobic compounds, as PAHs. In this study, we focus on the capacity of rhizomolipid biosurfactants, produced by Pseudomonas aeruginosa 19SJ, to enhance the bioavailability of different soil-sorbed 14C-labeled PAHs in soil aqueous systems. In a well-controlled batch system, we studied the effect of biosurfactants, at concentrations above the critical micellar concentration (CMC), on the bioavailability of fast and slowly-desorbing fractions of 14C-labeled PAHs, present in soil. Desorption kinetics of 14C-PAHs from soil were performed by Tenax solid-phase extraction and compared to mineralization kinetics by a PAH-degrading soil bacterium (Micrococcus gilvum VM552) in the presence of biosurfactants. Results indicated that the promoting effects of biosurfactants on biodegradation were accentuated in soils exhibiting a slow-desorption profile. Given the biodegradable and nonbiocidal nature of biosurfactants, their use constitutes a promising alternative for promoting bioavailability of this ‘resistant’ fraction in a sustainable way.

TU 290

Bacterial taxa and sustainable remediation: effects on bacterial dispersal

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We studied the effect of organic compounds on the movement of bacteria in porous media as a bioavailability-promoting strategy. The positive effects of -form energy-consuming - mobilization approaches in bioremediation depend on the efficiency of bacterial movement in porous media, which is often restricted by high deposition rates and adhesion to soil surfaces. Besides, adhesion to surfaces may be sometimes beneficial in promoting biodegradation of slowly-desorbing chemicals. In well-controlled batch systems, we assessed the potential of organic compounds on the motility behaviour of Pseudomonas aeruginosa and on the bioavailability of 14C-labeled PAHs. The results indicated that the movement behaviour of bacteria can be affected by the presence of organic compounds, as well as microbial dispersal. Moreover, the motility behavior could be affected by organic compounds, which could be used to promote bacterial dispersal, thus enhancing an enhanced and sustainable bioremediation performance.

TU 291

Behavioural responses of Tetrahymena pyriformis exposed to microgradients of hydrophobic organic chemicals

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Behavioural changes of microorganisms may reveal sublethal adverse effects of hydrophobic organic chemicals. Using them as an endpoint requires, however, that the organisms can be observed microscopically during exposure. We therefore applied passive dosing on microscope slides as a new experimental platform to study the effect of selected polyaromatic hydrocarbons (PAHs) on the behaviour of the ciliate Tetrahymena pyriformis. Motile cells and organisms that are able to sense chemical signals may direct their movement along concentration gradients of a chemical, a behaviour known as chemotaxis. T. pyriformis was therefore exposed to various PAH-gradients and the swimming behaviour of the cells was then recorded under infra-red light illumination. Trajectories of the cells were obtained using an automated cell tracking software and were subsequently analyzed for chemotaxis by statistical measures. The Taylor model, which mathematically describes the scale-dependent transition from ballistic to diffusive movement, was used to calculate the significance of the movement and the swimming behaviour of the cells. The results did not reveal chemotactic behaviour of T. pyriformis in the concentrations gradients of the compounds tested. However, motility behaviour was affected at levels that were about two orders of magnitude below the reported effective chemical activity causing 50% lethality, emphasizing that behavioural responses are a sensitive endpoint in toxicity testing.

TU 292

Sorption of ionized and polar contaminants to natural dissolved organic matter

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Concentrations of dissolved organic matter (DOM) have been rising in the environment over the past decades due to climate change and decreases in acid rain. The transport potential of DOM in the environment, and the partitioning of DOM concentrations in surface water. It is therefore relevant to gain more insight into the mechanism of organic contaminant sorption to DOM. In this study, the interactions between polar organic contaminants (neutral and ionized forms) and DOM were studied to assess the
influence of charge and molecular structure on the sorption process. Representative emerging contaminants were selected, covering hormones, pharmaceuticals, personal care products, and pesticides. Batch sorption studies were conducted with natural DOM-containing water samples obtained from Finland, United Kingdom, and the Netherlands and by using polyacrylate passive samplers to determine aqueous contaminant concentrations. The DOM samples were characterized by chemical and physical fractionation methods. The effect of charge and molecular structure on the sorption to both DOM and polyelectrolytes was investigated. Using polyacrylate as a positive charged polyelectrolyte, positively charged DOM showed a stronger pH-dependent sorption to DOM, whereas the neutral forms were the most important species responsible for sorption to polyelectrolyte. Negatively charged DOM showed the lowest affinity to both DOM and polyelectrolyte. For most neutral contaminants investigated, the water phase represented a more favorable medium compared to DOM. The obtained results may increase our knowledge of the sorption of polar organic contaminants to DOM, which is currently based on only limited data.

TU 293

Binding can improve the mobility and uptake of hydrophobic organic compounds V. Goulariumou1, K.E.C. Smith1, L.W. de Jonge1, C. Collins2, P. Mayer1

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The binding of hydrophobic organic compounds (HOCs) is usually considered to be a risk reduction process. However, if the bound form becomes mobile then it can in fact enhance uptake and risk. This phenomenon can be seen in 1) colloid facilitated transport 2) the use of chemical agents to enhance soil remediation and 3) in biology, where body fluids bind HOCs, thus reducing their bioavailability.

In the current work we studied the above examples using enhanced capacity (E) as a new measurement endpoint. Enhanced capacity (E) has some similarities to the well-established concept of “solubility enhancement”, but should not be confused with it. Solubility enhancement is determined at, and applies only to, the saturation level of the aqueous solution of the particular compound that is to be sorbed. E, on the other hand, can be measured at any level in vitro or in vivo, with different species, and it is generally a quantitative measure of bioaccessibility. E in a defined aqueous solution can be measured by adding crystals of HOCs into an aqueous solution, and to due the low relative standard deviation of the method even small enhancements could be measured with high precision.

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TU 294


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Biodegradation plays a key role in PAH fate, and understanding kinetics as a function of (bio)availability is critical for elucidating their persistence. However, biodegradation mainly occurs in an aqueous environment, posing technical challenges for producing reliable kinetic data because of low PAH solubilities and sorptive losses. To overcome these, an experimental approach based on dynamic passive dosing is presented which avoids using co-solvent for introducing the PAHs, buffers substrate depletion so biotransformation is measured within a narrow and defined concentration range and enables high compound turnover to simplify biodegradation measurements even at realistically low concentrations. The biodegradation kinetics of two PAHs by the bacterium Sphingomonas paucimobilis EPSA05 were measured at defined dissolved concentrations in the range of 100 ng L-1 to 24 µg L-1 for fluoranthene. Both compounds had similar biodegradation rates, and these increased in line with higher dissolved concentrations. First-order biodegradation rate constants were similar for both, but these decreased at higher dissolved concentrations. The experimental results were compared to those obtained using different modelling approaches, including simulations based on the Best equation. These showed a good agreement at the lower dissolved concentrations, whereas at higher concentrations the experimental measurements were lower than predicted, and indicate that other factors such as PAH toxicity or essential nutrient availability play a role. Therefore, combining measurements of PAH biodegradation kinetics at defined concentrations using dynamic passive dosing, and their interpretation by modelling is a useful tool to further understand their bioavailability, biodegradation and persistence.

TU 295

Enhanced mass transfer of hydrophobic organic compounds from NAPLs into the aqueous phase by dissolved organic carbon K.E.C. Smith1, M. Thullner1, L.Y. Wick2, H. Harns2

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Hydrophobic organic compounds (HOCs) are often found as mixtures in the form of non-aqueous phase liquids (NAPLs). Due to their hydrophobic nature, the HOCs preferentially remain in the NAPL with slow mass fluxes into the aqueous phase. However, since water dissolved HOCs play a key role in diffusive uptake into organisms, microorganisms using HOCs as a source of carbon and energy face a large reservoir of inaccessible food in the NAPL which is often reflected in slow bioremediation of NAPL contaminated sites. Interestingly, mobile “colloidal-like” phases can contribute to diffusive mass exchange processes between surfactant and the bulk aqueous phase, a phenomenon termed enhanced or facilitated diffusion. Therefore, this study investigated the role of dissolved organic carbon (DOC) in enhancing the mass transfer of hydrophobic organic compounds from NAPLs into the aqueous phase above that attributable to dissolved molecular diffusion alone. In controlled experiments, mass transfer rates were increased by up to a factor of four in the presence of DOC, with the greatest enhancement being observed for more hydrophobic compounds and highest DOC concentrations. These increases could not be explained by dissolved molecular diffusion alone, and point to a parallel DOC-mediated diffusive pathway. The nature of the DOC-mediated diffusion pathway was investigated using DOC concentration-diffusion-based models, and found to increase with DOC concentration and compound sorption. Therefore, for “super” hydrophobic compounds this pathway could both dominate but also increase mass transfer rates by orders of magnitude, even at environmental DOC concentrations. This has important implications for their bioremediation, as well biotransformation and toxicity.

TU 296

On the effect of vitamins and nutrients on the solubilization of petroleum/biodegradable blends in water M.H. Oosterhuis1, M. Suidan1,2, A. Venosa1

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The effect of mineral salts on the aqueous solubility of non-electrolytes is largely assumed to exhibit the “salting out” or “salting in” effects in which the aqueous solubility of non-electrolytes is decreased or increased upon the addition of salts. Pioneering investigations by Sestchenko [1] and later by Carter and Hardy [2] among others, resulted in semi-empirical relationships that describe the dependence of the solubility of non-electrolytes solutes on salts concentrations. We conducted equilibration experiments with petroleum/biodegradable blends (B0, B20, B40, B60, B80, and B100, where B100 = 100% biodiesel) in water in the presence and absence of standard vitamins and nutrients used for bacterial growth studies. The solubility of aromatic compounds was found to be in agreement with the salting out effect in the presence of the added nutrients. The solubility of aromatic compounds in the presence of B100 is significantly lower than in the absence of nutrients. The solubility of aromatic compounds in the presence of B100 was found to be significantly enhanced (p < 0.005), up to 20-fold in the presence of the fatty acid methyl esters (FAMEs) and the vitamins and nutrients medium, compared to FAMES and deionized water alone. A similar observation was made by Baker [3] regarding the effect of salts on solubilization of the alkanes and aromatic compounds in the presence of fatty acid soap solution. They reported that, in the presence of sodium chloride, the solubility of aromatic hydrocarbons in dilute fatty acid soap is markedly higher than the presence of organic hydrocarbons is lower. Those observations are found to contribute significantly to the bioavailability of the n-alkanes in the water column. Biodegradation studies conducted in our laboratory showed significant enhancement for the microbial utilization rates of the n-alkanes in the petroleum/biodegradable blends, which we interpret to be due to the increase in aqueous concentrations of the n-alkanes in the presence of the FAMES and nutrients.

References

TU 297

Photo-transformation of 2,3,7,8-TCDD in presence of natural organic matter studied by in vitro bioassays M.P. Roberts, B. Goei1, L. Bahl1,2

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as a representative of hydrophobic organic compounds (HOCs), frequent anthropogenic environmental pollutants comprising also various pesticides, can cause many adverse effects in organisms. Activation of arylhydrocarbon receptor (AhR) can be very important mechanism of toxicity for so-called “dioxin-like” HOCs. Natural organic matter (NOM), being naturally occurring compounds (up to 50 mg/L in waters), occurs together with HOCs in contaminated water. Besides other important ecological properties, NOM serves as a natural source of reactive oxygen species that are formed after NOM irradiation. Direct photolysis of HOCs is a very important way of their degradation in the aquatic environment. Nevertheless, oxygen species, formed after HS irradiation, can theoretically enhance the photochemical degradation of HOCs. In our previous study, we have assessed the ability of various NOM concentrations to enhance photo-degradation of TCDD. Aqueous TCD+HOCs
solutions were irradiated by sun light in quartz tubes for up to 3 days. Photo-degradation of TCDD studied by in vitro assay (based on the HHIEC-luc transgenic cell line) was observed, but both TCDD alone and TCDD in mixtures with low concentrations of NOM (up to 10 mg/L) were photo-degraded in almost the same half-lives. High NOM concentrations (25-150 mg/L) have significantly extended the half-lives of TCDD photo-degradation, probably due to filter effect of more coloured solutions. Supported by project CETOCOEN (European Regional Development Fund no. CZ.1.05/2.1.00/01.0001).

TU 298 Influence of sorption on bioavailability and biodegradation of secondary alkane sulfonates (SAS) in marine sediments

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Concentrated aqueous solutions of SAS, often used by the pesticide industry or accidental spills. Time weighted composite samples of column influent and effluent were analysed for X-RCM and DOC and treatments with and without concentrated aqueous saccharose solution to achieve elevated DOC and to simulate the pollution of the Rhine with low molecular organic compounds, e.g. from food industry or waste water discharges from surrounding populations. Surfactants, with a worldwide production over 10 million tons per year, are among the organic contaminants showing highest concentrations in wastewater. Most available studies on this topic deal with the distribution and fate of alkylphenol polylvethoxylates (APEO) in aquatic systems, as some of their degradation intermediates are endocrine disruptor compounds (EDC). Linear alkylbenzene sulfonates (LAS) have also been detected in their natural environment, as they show up in the worldwide production volumes. Environmental data on many other surfactants, however, are still scarce. This is the case of secondary alkane sulfonates (SAS), one of the major anionic surfactants used in the market of dishwashing, laundry and cleaning products. Although this compound is easily removed during wastewater treatment, previous studies have shown that SAS and other anionic surfactants such as LAS accumulate in sludge as they are hardly biodegradable in anaerobic digestion. Recently, aerobic biodegradation of SAS was confirmed in marine sediments due to the presence of sulfate reducing bacteria. This research has focused on determining whether SAS are biodegradable or not in absence of oxygen in the marine environment, and, if that happens, on the role of sorption on the speed of the biodegradation. First sorption experiments were performed using several amounts of sediments (0.5-5 g) and SAS concentrations (1-10 mg/L). Distribution coefficients (Kd) of 1.550 L/Kg were observed. It has been observed that the sorption capacity is much higher for those homologues having longer alkyl chains (e.g., C17-SAS) rather than for more polar homologues such as C14-SAS. Later, biodegradation experiments using anoxic marine sediments and seawater were conducted. SAS aerobic biodegradation was observed for the first time, reaching overall values up to 98% in 166 days. Half-life values ranged from 20 days (C14-SAS) to 37 days (C17-SAS), showing that the speed of this process significantly depends on the sorption capacity and, therefore, bioavailability, of each SAS homologue.

TU 299 Remediation of PCB- and PAH- contaminated soil with modified clays

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Sodium dodecyl sulfate (SDS) and humic acid were used to modify bentonite. Kaolinite was modified by Papaya Seed. Different percentages of the modified clays were mixed with the contaminated soil and suspended in 0.01M CaCl2 in the dark for twenty days. After equilibration analysis of the PCBs and PAHs in solution was carried out using SPME coupled with GC/MS. Presented are the sorption capacities of the modified clays and results of remediation. The results of this study indicate a promising potential of the modified clays to immobilize PCBs and PAHs in soils.

TU 300 Fate of iodinated X-ray contrast media in a soil column percolation experiment simulating elevated dissolved organic carbon (DOC) by amendment of saccharose

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The Japanese Positive List System for Agricultural Chemicals was implemented in 2006. Under this system, the uniform limit was set at 0.01 mg/kg for agricultural chemicals for which maximum residue limits (MRLs) have not been established. Since then, numerous crops at levels above the uniform limit, and the distribution of these crops has been prohibited. One of the reasons why the limit has been exceeded is that succeeding crops are contaminated by pesticides remaining in the soil. Assuming that these pesticides in the soil are taken up by plants via the soil solution, an understanding of the soil-water distribution of the pesticide in the crops is strongly influenced by soil organic matter content. However, there is little information available on the influence of soil pH and electrolyte concentrations, changed by chemical fertilizer treatment, on pesticide adsorption. We therefore used batch experiments to investigate the effects of these factors on the partition coefficient for adsorption (Kd) of about 30 pesticides. We used buffer solutions (pH 3, 5, 7.5, and 9) and soil solutions with different organic matter contents. Each soil was shaken with an aqueous solution containing 0.1 mg/L of pesticide at a soil-to-solution ratio of 1 to 5, with a 24-h equilibration period for all experiments. Increasing the CaCl2 concentration from 0.01 to 0.1 mol/L had a significant on effect soil adsorption of pesticides, but the Kd values with 1 mol/L CaCl2 solution were slightly higher than those at the other two concentrations. Pesticide adsorption was pH dependent. In general, Kd values were negatively correlated with pH levels, and the rate of Kd change, calculated by dividing the Kd values by the pH values at pH 3 and pH 9, was greater in the case of soils with higher organic carbon contents and of pesticides with higher octanol/water partition coefficient (log Kow). The results suggest that the hydrophobic interactions between pesticides and soil organic matter are influenced by pH levels. Further, it is implicated that crops can take up the pesticide from soil at high pH compared with at low pH.

TU 301 Effect of pH and electrolyte concentration on soil adsorption of pesticides

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The positive list system for pesticide residue in food, such as crops, was applied in Japan in 2006. For pesticides for which maximum residue levels were not set, the residue levels in food must not exceed 0.01 mg/kg (uniform limit). Pesticides, which are applied to foliage and soil at the crop cultivation, remain in soil after harvest, and pesticides remaining in soil may contaminate the succeeding crop. Crops, for which growing cycle is short, are rotated in Japan. Therefore, the residue levels of some pesticides in succeeding crops have exceeded 0.01 mg/kg in Japan. To prevent excess of the residue level 0.01 mg/kg in the succeeding crop, the succeeding crop must be cultivated in consideration of the physiophysical properties of the pesticide applied to the preceding crop, 2) the soil properties affecting the pesticide residue in soil, and 3) the pesticide's uptake into the succeeding crop. The succeeding crop may take up the pesticide in soil solution from the root or the stem as a kind of a pesticide's uptake into the crop. Data on the distribution of a pesticide in soil/solution (pesticide soil adsorption) is important for assessing the risk of pesticide contamination of the succeeding crop. Soil adsorption of nonionic pesticides

TU 302 Relationships between soil adsorption of pesticides and pesticide/sol/soil properties

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1Food and Agricultural Materials Inspection Center, Kodaira, Tokyo, Japan

The positive list system for pesticide residue in food, such as crops, was applied in Japan in 2006. For pesticides for which maximum residue levels were not set, the residue levels in food must not exceed 0.01 mg/kg (uniform limit). Pesticides, which are applied to foliage and soil at the crop cultivation, remain in soil after harvest, and pesticides remaining in soil may contaminate the succeeding crop. Crops, for which growing cycle is short, are rotated in Japan. Therefore, the residue levels of some pesticides in succeeding crops have exceeded 0.01 mg/kg in Japan. To prevent excess of the residue level 0.01 mg/kg in the succeeding crop, the succeeding crop must be cultivated in consideration of the physiophysical properties of the pesticide applied to the preceding crop, 2) the soil properties affecting the pesticide residue in soil, and 3) the pesticide's uptake into the succeeding crop. The succeeding crop may take up the pesticide in soil solution from the root or the stem as a kind of a pesticide's uptake into the crop. Data on the distribution of a pesticide in soil/solution (pesticide soil adsorption) is important for assessing the risk of pesticide contamination of the succeeding crop. Soil adsorption of nonionic pesticides
is influenced by organic carbon content in soil; however, there are few investigations on influence of pesticide and soil properties except organic carbon content on the adsorption of pesticide in soil.

The present study conducted batch experiments with approximately 30 pesticides and 7 soils in Japan to determine their soil adsorption coefficients (Kd values). The results of relationships between Kd values and pesticide/soil properties will be presented.

TU 303
Leaching of aged DDTS and current use pesticides in undisturbed soil columns: non-ionic surfactant and carbamoyl acids effects
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1Faculty of Technical Sciences, Novi sad, Serbia
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Soil pollution by Persistent Organic Pollutants such as DDTS (p,p’-DDT and its metabolites p,p’-DDE and p,p’-DDD) represents a continuous source to the environment. As a consequence of their high persistence DDTSs occur up to 60 cm on agricultural soils. The Current Use Pesticide (CUP) endosulfan is characterized by its high lipivixation potential and may be transported by surface runoff, low Kow values and leaching. The adsorption potential of soils is affected by many factors such as soil type and hydrology, climate, and climatic events such as plant growing may modify pesticide availability and leaching. Surface waters receive discharges from industrial, urban and agricultural uses that will account for a variety of compounds of soils, surfactants and dissolved organic carbon. This work studies the vertical distribution and movement of aged DDTSs and endosulfan in undisturbed soil columns irrigated with: 1-control (riverine water), 2-Tw-80 (riverine water + Tween 80 0.5 cmc), and 3-acids (riverine water + sodium citrate and sodium oxalate, 0.05 M).

Experiments were carried out with 13 x 30 cm soil columns taken from Aridisol soils from a fruit field in Patagonia, Argentina. Columns were saturated with distilled water and solutions (4L) were added under saturated condition. One L leachates (nw=4) were collected for pesticide residues analysis. After elution, columns were dried (24 h), opened and analyzed for soil structure and mineralogy. The obtained results were interpreted by means of batch technique and source analysis. Pesticides on soil and water by GC-MS, p,p’DDE and p,p’DDD concentrations were determined by GC-MS-MS. The peak retention time on GC-MS-MS was similar to the technical mixture which is widely used in the region. In post-flood muscle, DDTs levels increased 5-fold than pre-flood. The metabolite p,p’-DDE represented the highest increase in post-flood liver (up to 400 times) and difference between post- and pre-flood fish (up more than 240 times) was the main compound on the first elution volume and p,p’-DDE started to lixiviate after 2 L while for Tw-80, DDE represents the main residue in all elution volumes.

TU 304
Degradation of UV filters in sewage sludge by the linyinolic fungus Trametes versicolor and study of the 4MBC degradation process in liquid medium
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Sunscreens agents, also known as UV filters, have become very popular chemicals since they were shown to have a protective role against photoaging, photocarcinogenesis and photoimmunosupression. They are extensively used in personal care products but are also present in a wide variety of industrial goods such as paints, paints, or plastics to prevent photodegradation of polymers and pigments. UV filters can enter the environment through the liquid effluent of wastewater treatment plants (WWTPs) but also absorbed in the sludge due to their high hydrophobic character, and later spread on agricultural fields. A solid-state treatment of WWTP sludge is not an option due to the high cost of the process. Under controlled conditions, Tw-80 and Acids, o-endosulfan was the main compound on the first elution volume and p,p’-DDE started to lixiviate after 2 L while for Tw-80, DDE represents the main residue in all elution volumes.

TU 305
Soil pollution by Persistent Organic Pollutants such as DDTs and its metabolites
M. Miloradov1, M.M. Miloradov1, J.S. Slobodnik1, M.D. Djogo1, M. Milovanovic1, J.R. Radosevic1, M.M. Turk Sekulic1
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Soil pollution by Persistent Organic Pollutants such as DDTs and its metabolites represents a continuous source to the environment. The environmental fate of these compounds depends on their physicochemical properties, which are strongly influenced by soil and sediment characteristics and their physical and chemical properties, which are strongly influenced by soil and sediment characteristics and their physical and chemical properties. In addition, sorption affinity to five different Eurosoils was tested, which ranged in organic carbon content (fOC) from 1.3 - 9.2%, and in clay content from 6 - 75%. We examined what type of sorption to natural soils can be predicted based on sorption to industrial soil components alone. In general, sorption of primary amines to clay minerals was weak relative to quaternary ammonium compounds (quats), whereas sorption of primary amines to SOM was strong relative to quats. Such relative sorption affinity difference between test compounds between SOM and clay minerals was used to evaluate soil sorption.

TU 306
Concentration levels of phthalates in water and sediment samples from Nadela river basin, Vojvodina region
M.B. Miloradov1, M.M. Miloradov2, J.S. Slobodnik1, M.D. Djogo1, M. Milovanovic1, J.R. Radosevic1, M.M. Turk Sekulic1
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Phthalates belong to the group of dominant industrial pollutants and are ubiquitous over the water bodies with low water solubility/high fat solubility and low volatility. Due to their low water solubility, the phthalates hydrolyse relatively slowly, but the actual dynamic rate varies according to temperature, concentration and solution alkalinity. The environmental properties of phthalates have much impact on their distribution in the environment. Among the phthallates diethylhexyl phthalate (DEHP) predominates and has many possible applications, especially in PVC. The highest concentrations of DEHP are found in products for flooring, building and plastic coated fabrics. Phthalates are also included as plasticisers for binders in different kinds of paints and adhesives. Within the Project the concentration levels of phthalates in riverine water on polluted soils during irrigation or flooding events as well as plant growing may influence the occurrence of phthalates in soils. The obtained results were interpreted by means of batch technique and source analysis. In addition, sorption affinity to five different Eurosoils was tested, which ranged in organic carbon content (fOC) from 1.3 - 9.2%, and in clay content from 6 - 75%. We examined what type of sorption to natural soils can be predicted based on sorption to industrial soil components alone. In general, sorption of primary amines to clay minerals was weak relative to quaternary ammonium compounds (quats), whereas sorption of primary amines to SOM was strong relative to quats. Such relative sorption affinity difference between test compounds between SOM and clay minerals was used to evaluate soil sorption.

TU 307
Concentration levels of PBDEs, PCBs and organochlorine pesticides in fish following a mega-flooding episode in the Negro River basin, Argentinean Patagonia
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2CONECO, FURG Universidade do Rio Grande, Rio Grande do sul, Brazil

A mega-flooding event in the Negro River basin, Argentinean Patagonia, caused a significant outflow of contaminants from the surrounding soils. Agriculture, hydroelectric central and industrial-urban activities are the most important activities in this basin. This study evaluates the effects of flooding events on the levels of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), endosulfan, DDTS and polychlorinated biphenyls (PCBs) levels in females of whitebait (Osontexushi hatchers) tissues. Muscle, liver, gills, gonads and stomach content were analyzed by GC-MS. Levels in water, suspended particulate matter (SMP) and sediments (SS) were also measured. Post- and pre-flood fish showed the following patterns of PBDEs, PCBs and organochlorine pesticides (OCPs): PBDEs levels in post-flood fish increased until 23 times (from 63.2 to 1.478.6 ng lipid in liver), which were dominated by penta- (PCB-110, 118) and hexa- (PCB-138, 139) congeners. These results could have stemmed from historical usage of Aroclor 1254 and 1260 in Argentina. Post- and pre-flood muscle, DEHS levels increased 5-fold than pre-flood. The metabolite p,p’-DDD represents about 40% of the total DEHS concentrations. A significant decrease would be the result of the intensive use of this insecticide during long time on agricultural practices. Contaminant profiles observed in pre- and post-flood silverside were according to water, SPM and SS, showing that this species is a good biomonitor of aquatic pollution of Negro River. The presence of pollutants in this river was modified and enhanced by the flooding, increasing their bioavailability to fish. Additionally, pollutant occurrence deserves more attention, and monitoring programs are recommended in order to diminish their incorporation to aquatic ecosystem.

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**TU 308**

Polychloro-dioxins, furans and biphenyls in fish, crabs and clams from the San Jacinto River Waste Pits, TX

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United States of America

Fish, crabs, and clams were collected from the San Jacinto River waste pits, a superfund site in Houston, TX and analyzed for polychlorodibenzo-p-dioxins, polychlorodibenzofurans (PCDD/Fs) and dioxin like polychlorobiphenyls (dl-PCBs). Sample preparations comprised of tissue homogenization, enhanced pressurized liquid extraction (PLE), aqueous two phase partitioning, heat treatment and sequential extraction and were followed by purification and gas chromatography/mass spectrometry (GC/MS). The method detection limits ranged from 2.0 to 10 pg/g ww in tissue samples. Ten out of twelve priority dl-PCBs (Identified by World Health Organization, 2005) were measured with concentrations ranged from 2.0 to 948 pg/g ww. Average concentrations were at least an order of magnitude higher than that of PCDD/Fs. Average PCDD/Fs fish tissue concentrations were at least an order of magnitude than that of crabs and clams. TEQfound was to be at least 25X higher than TEQfish. Biomagnifications factors and Biota Sedimentation Accumulation Factors were also calculated.

**TU 309**

Effect of humic substances on remediation of soil: phosphogypsum mixtures

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Phosphogypsum (PG) is a major by-product of industrial production of phosphoric acid. Due to high content of nutrients (calcium, phosphorus and sulfur) it is sometimes used as a fertilizer for poor unfruitful soils. But besides nutrients, PG also contains toxic impurities, such as fluorine, strontium and some others. One of contemporary tools to reduce the ecotoxicity of polluted soils and soil-pog samples is application of humic substances (HS). The objective of this study was to evaluate the detoxifying ability of a number of humates towards PG applied to model soil mixtures.

Model soil (MS) contained (% wt): 20% of kaolinite, 10% of peat and 70% of sand with particle size 0.2-0.4 mm (ISO 11268-1). PG was carefully mixed with MS at concentration 3.3 and 7.5 wt.%, %, Hymates from peat - Pe-FlexK, Pe-EcoK, coal - BC-EnK, BC-HumNa and lignosulfonate - OW-LHK introduced into the MS in the form of an aqueous suspension (distilled water) to achieve a concentration in soils 0.005 and 0.020 wt. %. The ecotoxicity was evaluated in water extracts using three species of test-organisms from different taxonomic groups: microalgae Scenedesmus quadricauda (decrease of cells population growth, 72h), crustaceans Daphnia magna (mortality, 96h), and higher plants Sinapis alba (root of decrease elongation, 120h). Toxicometric parameters (EC50, median PG concentration that caused a 50% test-reaction reduction, and NOEL - no observed effect level - the PG-concentration that caused test-reaction reduction below the toxic level) and detoxification indexes D were calculated.

Analyses of average detoxification indexes DAV (averaged over three test-organisms) showed that influence of humates depended both on PG concentration and humates’ nature. Thus, application of PG at concentration 7.5 wt.%, %, was toxic for all the test-cultures at all treatments and humates were not able to decrease the toxicity. At lower concentration of PG 3.3 wt.%, %, some humates decreased the toxicity (DAV = 0.5-0.8), whether other did not. Among all humates tested the highest detoxifying ability was observed for two samples: Pe-FlexK from peat and BC-EnK from coal. For these humates EC50 and NOEL values increased in 1.3-1.8 times for D. magna, in 1.5-1.8 times for S. alba, and 1.15-1.4 times for M. Sinapis.

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bacteria, the inhibition of the Enoyl-ACP-reductase during the fatty acid elongation [McMurry et al., Nature, 1998].

Identification of lipophilic metabolites with the NIST and Golm library for GC-MS data revealed many fatty acids (e.g. hexadecanoic acid, oleic acid, octadecanoic acid) changing their concentrations since they perform numerous functions. Their multifunctionality, the vast surface of exposure to the external media and their location in the fish body, justify their importance as key organs for the direct action of pollutants in the aquatic environment. This work aimed to assess biochemical and morphometric changes in fish, after exposure to several compounds, including pesticides, detergents and pharmaceuticals, which can be quantified using appropriate analytical tools. The results of this study revealed that not only neostigmine significantly decreased the activity of GSTs at concentrations of 1 and 100 µmol. Considering the morphometric analysis of the gills, the data obtained showed that chlorfenvinphos increased values of BET and decreased SLL at a concentration of 3.9 µg/L. The drug neostigmine was responsible for an increase in SLL values at the highest tested concentration, and pyridostigmine increased SLW values at the concentration of 100 µmol/L. The potential action of neostigmine in exposed fish, with inhibition of GSTs activity, is a noteworthy result, since it establishes a reduction in the effectiveness of conjugation and elimination of other xenobiotics, enhancing toxicity. Principal component analysis showed a surface respiratory disturbance as result of exposure to several xenobiotics, which could be advantageous for fish by reducing toxicant absorption.

Oxidative stress and higher level effect of engineered nanomaterials in the nematode Caenorhabditis elegans: functional toxicogenomic approach

Relationship between exposure duration, mortality, bioaccumulation and physiological parameters in glochidia (larvae) and juvenile freshwater mussels exposed to copper

Physiological and biochemical biomarkers in the catarina scallop Argopecten ventricosus (Sowerby, 1842), as tools to Monitor in the Ensenada de la Paz B.C.S., México.

In this study an evaluation of 4 biomarkers: respiration rate, O:N index, oxidative stress and AchE activity were carried out in the gill tissue of pearl oyster, from the cultivation vivero, to detect presence of toxic and genotoxic substances, and evaluating the use this biomarkers as reliable tool in environmental biomonitoring studies. Studies of toxicity (5.15 ± 0.58 cm; 1.21 ± 0.10 g) and one of the unique harbours (2.18 ± 0.10 cm; 0.07 ± 0.02 g) in the experiment were used. The results of this study showed that the most sensitive biomarker to copper pollution. In this respect, this study aimed to characterize the acute (96 h) and chronic (28 days) sensitivity in newly released glochidia larvae (L. cardium) and 6 months old juvenile (L. siliquoidea) freshwater mussels exposed to copper and understand the mechanism(s) of action. Dissolved EC50 and EC20 for glochidia larvae was 22.7 and 9.7 µg Cu/L, respectively. These values indicated that L. cardium are protected by the BLM-based acute WQC 2.3 µg Cu/L but under protected by the hardness-based WQC 113.11 µg Cu/L. Glochidia copper uptake and accumulation indicate that there is a biodosification process based on physiochemical interactions between metal and functional groups of cell membrane which can cause damage at the cell surface. Our results also showed that a decrease in whole body sodium concentration. Most striking in the chronic experiment was the reduction of whole body sodium content and inhibition of Na+ -K+ ATPase, indicating copper toxicity for mussels chronically exposed is a consequence of an ionoregulatory disturbance. In contrast to the acute experiment, redox oxidative stress was not associated with any biochemical indicators. Most striking in the chronic experiment was the reduction of whole body sodium content and inhibition of Na+ -K+ ATPase, indicating copper toxicity for mussels chronically exposed is a consequence of an ionoregulatory disturbance. In contrast to the acute experiment, redox oxidative stress was not associated with any biochemical indicators.

Copper - are they comparable in their sensitivity towards chemical stress?

Cryptic lineages - are they comparable in their sensitivity towards chemical stress?

Species richness and population density of freshwater mussels Unionidae have declined substantively throughout North America. Surveys have revealed that environmental pollution is one of the most important causes. That is the reason why understanding the life cycle of these aquatic species and the biological impact of the pollution is crucial. A number of factors contribute to the protective role of the bivalves in their environment. In these circumstances, it is not unusual to find a high diversity of chemical forms in the aquatic environment. Despite the large number of studies that have been done, the relationship between higher level effects and the mechanisms of toxicity. Current mechanistic studies on ENM have reported oxidative stress reaction, as one of the most important mechanism of toxicity. In this study, to understand the relationship between higher level effect of ENM and mechanism of toxicity, functional toxicogenomic analysis was performed in the nematode Caenorhabditis elegans. Survival and neurodegeneration of the gonads were examined in worms exposed to silver nanoparticles (AgNPs) and multi-wall carbon nanotubes (MWCNT). Subsequently, mechanism of toxicity was investigated using microarray followed by gene ontology (GO) and pathway analysis. Finally higher level effect of altered genes and pathways selected as mechanisms of toxicity was investigated using functional genomic tools such as, loss of function mutant or RNAi of genes involved in these pathways. Their responses were compared with those of wildtypes.

Copper, the inhibition of the Enoyl-ACP-reductase during the fatty acid elongation [McMurry et al., Nature, 1998].
Accordingly, the present study investigated two cryptic *Gammarus fossarum* lineages - namely type A and type B - for differences in their sensitivity towards chemical stress. Two size classes of each cryptic lineage type were exposed to different concentrations of the fungicide tebuconazole or the insecticide thiacloprid in six independent experiments. The feeding rate on leaf discs was determined after seven days of exposure as a measure of sensitivity. The data was then analyzed by combining meta-analysis with the cryptic lineage type A compared to type B (n=16; p<0.0001), by combining the results of all bioassays. Discrete meta-analyses for each of the chemical tested, showed a similar tendency, however, results were statistically significant only for tebuconazole (n=8; p<0.0001). This deviation in sensitivity between lineages is new due to the minor consideration of the cryptic lineages concept in the field of ecology. Moreover, it is relatively unexplored. Hence, further studies should address the sensitivity to other stressors, by also considering ecological aspects like the physiological fitness, of a broader range of cryptic complexes. Nonetheless, genetically distinct lineages may confound the results of ecotoxicological tests, as indicated in the present study, and should thus be interpreted with caution.

**TU 322**

**Status of PAHs in greater Johannesburg area and possible sources**

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The presentation gives an overview of the status of the presence and levels of PAHs in greater Johannesburg area. Although many studies of persistent organic pollutants such as pollutants of the alkylic perfluorinated compound family have been in South Africa, little has been done in Johannesburg area. So the study on PAHs is still having a lot of gaps. The presentation gives an overview of modern extraction techniques that have been used for the extraction of PAHs in water and sediments in rivers and dams in greater Johannesburg area. The common PAHs found and their concentration levels are presented. The levels of PAHs in water and sediments are not in different levels in those sediments. Detailed examination of the possible sources of PAHs is given and how these sources impact aquatic ecosystems in the studied area. Finally, the overview of PAH status in South Africa urban environment is given.

**TU 323**

**Linking transcriptome to phenotype: response of a mouse liver cell line to Benzo(a)pyrene**

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The aim of the systems biology initiative, “From contaminant molecules to cellular response: system quantification and predictive model development”, is to build a model of interactions between cells and the toxic chemical Benzo-a-Pyrene (BaP). As part of this initiative, we aim to identify the regulation of genes by BaP in a time and concentration dependent manner, to relate these to cellular dysfunction and predictability of the multiple toxic responses. Exposure to BaP has been linked to disturbances in cell cycle/growth, transcription and chromosome organization are negatively correlated to valproate levels. This study on PAHs is still having a lot of gaps. Genes related to toxicological effects have been enriched with functional terms such as structural molecule (cuticle/collagen), detoxification, phosphorylation and lipid metabolism. Genes related to the regulation of gene expression after BaP exposure are linked to different processes such as detoxification, cell cycle regulatory mechanisms and apoptotic responses. This suggests that the metabolism of BaP in Hepa1c1c7 cells is characterized by an acute phase induction of detoxification enzymes, which is associated with a downregulation of cell cycle regulatory genes. Indeed, lipid metabolism production is already observed after 4 hours of exposure to high BaP whereas no response is observed for the low BaP concentration. However, at 24 hours, ROS appears to be produced even for the low BaP concentration. Gene response after 12 hours of 5 uM BaP exposure moreover shows genes related to lipid metabolism. Indeed, lipid body accumulation using Nile red revealed significant changes in lipid droplet morphology already after 4 hours. We conclude that the low BaP concentration induces a small gene response, but that, together with biochemical defense mechanisms, helps the cell to return to its initial state. However, the high concentration causes strong pathway regulation and cellular dysfunction accompanied by early ROS production and lipid droplet modification.

**TU 324**

**Insights into the effects of silver by transcriptomics and proteomics in Chlamydomonas reinhardtii**

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Ecotoxicology aims to identify effects that toxicants elicit upon the environment. A major challenge is to understand the toxic mechanisms at a molecular level and how these relate to the organism and population level. Integration across various levels of biological organization, molecular, cellular and organismal, is required to draw conclusions on impacts in populations, communities and ecosystems [1]. Our research deals with the analysis of silver induced alterations in gene and protein expression in the algae Chlamydomonas reinhardtii and anchoring of these changes to specific cellular processes. The study involves multi-omics analysis, transcriptomics, proteomics, metabolomics and bioinformatics. The transcriptional profile of silver exposed algae was analysed using C. reinhardtii whole genome microarray. The global protein profile of silver exposed C. reinhardtii (5h exposure point) was done by Multidimensional Protein Identification Technology (MudPIT) which consists of 2D-IC and mass spectrometry. The transcriptome analysis determined that the most significantly regulated transcripts are those of metabolic pathways of photosynthesis, tetrapyrole synthesis, mitochondrial electron transport, protein transport and oxidative stress response. Similarly, the proteome profile showed these biological processes being significantly regulated indicating that silver affects key functional pathways in C. reinhardtii. The regulation of several transcripts/proteins involved in the photosynthesis and ATP synthesis could be correlated with inhibition of photosynthetic electron transport chain and ATP levels. Also, the regulation of these genes could be linked to the physiological endpoint of lipid membrane peroxidation and synthesis of storage lipid bodies. Global analysis of the transcriptome and proteome not only gives an insight into the mechanism of toxicity of silver but also, by linking them to ecologically relevant phenotypes, a better ability to predict environmental risk.

**TU 325**

**An integrative approach to understanding the response of Caenorhabditis elegans to valproate exposure**

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C.elegans has been extensively studied for the effects of chemical exposure, aging, pathogenicity and reproductive development. With a well annotated genome, well characterized regulatory cell line maps, ease of making knock-outs, availability of mutants and short life span, it is a good model system to study the effect of chemical exposure on organism. Recently, we treated wild type C. elegans with sodium valproate, a known teratogen and a histone deacetylase inhibitor used as a first line drug in epilepsy, manic depression, bipolar disorder and schizophrenia. Computational biology and omics technology was utilized to develop gene signatures associated with valproate exposure. We employed a systems biology approach by mapping transcriptomic data to known biological pathways to identify possible pathways affected by valproate treatment. The network models generated with the genes significantly associated with valproate indicates an overall downregulation of nuclear proteins. Metabolite signatures associated with valproate treatment were identified by Nuclear Magnetic Resonance (NMR) spectroscopy on formalin fixed worms. Functional analysis was done with DAVID gene ontology web tool. Genes were mapped to known pathways using Ingenuity Pathway Analysis software (Ingenuity® Systems, www.ingenuity.com). Networks derived from the genes highly correlated to valproate exposure were analysed by the same software. Metabolomic analysis was performed on the aqueous fraction of methanol/chloroform extracted samples on Brucker Avance 500MHz spectrometer at HFW-NMR Center, University of Birmingham. C.elegans were fixed prior to extraction, by adding 37% formalin directly on to plates. Treatment with valproate resulted in an increased body and a decrease in egg laying in a dose dependent manner. The genes with positively correlated expression to valproate were enriched with functional terms such as structural molecule activity (cuticle/collagen), detoxification, phosphorylation and lipid metabolism. Genes related to embryonic/larval development/reproductive development, cell cycle/growth, transcription and chromosome organization are negatively correlated to valproate levels. This could be due to the down regulation of the genes of the histone deacetylase complex, DNA replication initiation complex, splicingosome and some translation initiation factors by valproate.

**TU 326**

**Development of a resource for the collection, analysis, integration and interpretation of ecotoxicologically related high throughput data**

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In environment, the presence of chemical pollutants or climate changes can induce organism responses at the cellular and molecular level. Beside more traditional analysis systems like e.g. Western Blot of biomarkers, researchers can nowadays benefit of high throughput technologies (i.e. microarray, deep sequencing, 2-DE) that allow very large scale investigation. Data deriving from these technologies are often snapshots of the changes induced by the presence of environmental variations. Management, analysis and interpretation of the massive data obtained from high throughput technologies can be quite challenging and requires bioinformatics skills still beyond many laboratories interest. The aim of this project was to provide an exhaustive overview of the real cellular response and of changes in gene expression. Indeed they do not consider the relation between mRNA level, protein concentration and metabolite levels which are known to poorly correlate. Finally it would be very useful to compare the biological effects induced by the same environmental stress on different organisms. Hence, further studies should address the sensitivity to other stressors, by also considering ecological aspects like the physiological fitness, of a broader range of cryptic complexes. Nonetheless, genetically distinct lineages may confound the results of ecotoxicological tests, as indicated in the present study, and should thus be interpreted with caution.

We have benchmarked our platform with data obtained by high throughput analysis techniques - microarray and 2-DE - applied on the social amoebae Dictyostelium discoideum after treatment with several doses of mercury. We show the advantages of using our system which allows the comparison of the results obtained from the two techniques, effective tools for the visual inspection of data and integrates data from external resources such as specialized databases (e.g. genomic databases, metabolite databases, Gene Ontology).
More than 170 biological variables are screened, including microorganisms (e.g. biomasses of fungi and bacterial communities, bacterial diversity, enzymatic activities), information (data of different data quality, acute and chronic values, EC...)

The objective of the present study was to assess how the SSD approach can be extended to the use of the entire data sets of complex heterogeneous ecotoxicological data quantity, its use is currently restrained to a small number of compounds. One central problem is that commonly many available data are discarded from the analysis for reasons of limited validity, endpoint (exclusion of EC50, type data) and exposure time (exclusion of acute data). The objective of the present study was to assess how the SSD approach can be extended to the use of the entire data sets of complex heterogeneous ecotoxicological information. We present a coherent data quality, acute and chronic values, EC50, EC100, NOEC values). More precisely, we aimed to provide a method which provides reliable and robust PNEC assessment, which is applicable to wider area of compounds. For this purpose, we replaced the dichotomous classification of data as either valid or not valid by a system of quality scores which integrate on the inherent experimental and statistical reliability of data and on their biological relevance (organism and endpoint). This system serves as weighting factors of individual data in the SSD analysis. Furthermore, we studied different options of how to incorporate acute values. Different options (of probablistic Acute-to-Chronic ratios and weighting) for data treatment were integrated in a tool, which allows a generation of SSD functions and estimate uncertainties.

As a result, we calculated the standard SSD function (based on chronic NOECs only) for several real ecotoxicological data sets on compounds fulfilling the strict TG/D/REACH requirements for SSD application. Subsequently, we applied the developed method using weighting scores and acute to chronic extrapolation to the entire data set and bootstrap subsamples. SSD function obtained for subsamples of the entire data set using different options for data treatment were compared to the SSD based on chronic NOECs. Influences of different options for data treatment and sample size will be discussed with respect to the robustness and reliability of the SSD function and the associated HC value.

Investigating the replacement of NOEC and LOEC values with regression-based ECx values

The analytical strategy of the French "Bioindicators Programme" (2006-2012) is to (i) measure a large number of bioindicators in contrasted pedo-geo-climatic contexts (ii) apply a number of different static regression models to results arising from ecotoxicity tests, and to derive ECx values. The work described has shown that it is possible to apply a number of different static regression models to results arising from ecotoxicity tests, and to derive ECx values. The work indicates that, although not consistent for all datasets examined, it is possible to derive ECx values broadly similar to the respective NOEC and LOEC values. However, a number of issues remain which will be of importance when considering the possible replacement of NOEC and LOEC values with ECx values, including experimental design. It is thought that current test designs may not necessarily be suitable for data analysis using regression based methods, and that modifications to current protocols may be required to ensure that regression analysis of test data yields reliable and significant results.

The work described has applied regression analysis to a number of simulated data sets which were developed according to differing test designs. This has shown that the explanatory power of the regression analysis is strongly dependent on the quality of the data. However, the regression analysis has been shown to be impacted by experimental design. This could have implications for both the ethical and practical aspects of ecotoxicological testing, when seeking an optimum test design.

Statistical approach to select bioindicators for soil monitoring, risk assessment and soil characterization. Results from the French national programme 'Bioindicators'

The Random Forests is an asymptotic approach (Breiman, 2001), is used to classify, predict and select the best set of explanatory variables in large fields of research. Yet, only few applications were made in environmental research, especially in soil ecotoxicology. The statistical analysis of the French "Bioindicators Programme" (2006-2012) is to (i) measure a large number of bioindicators in contrasted pedo-geo-climatic contexts (ii) apply a number of different static regression models to results arising from ecotoxicity tests, and to derive ECx values. The work described has shown that it is possible to apply a number of different static regression models to results arising from ecotoxicity tests, and to derive ECx values. The work indicates that, although not consistent for all datasets examined, it is possible to derive ECx values broadly similar to the respective NOEC and LOEC values. However, a number of issues remain which will be of importance when considering the possible replacement of NOEC and LOEC values with ECx values, including experimental design. It is thought that current test designs may not necessarily be suitable for data analysis using regression based methods, and that modifications to current protocols may be required to ensure that regression analysis of test data yields reliable and significant results.

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A first step of statistical analyses, our results showed the importance of correlations between biological variables in intra and inter-groupings. Then discriminated analyses using Random forests revealed sets of relevant indicators for land use (crops, pastures, woods and forests), levels of metal contamination (high, medium and low) as well as...
TO 334
Advanced non-linear regression methods for concentration-response curves in R
S.J. Moe
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A recent document from the IEAM has suggested to ban the use of traditional no-observed-effect levels (NOELs) and lowest-observed-effect levels (LOELs) as basis for risk assessment and decision making, and instead use curve-fitting for estimation of concentration-response relationships. This should motivate an evaluation of current concentration-response modelling approaches in ecotoxicology, and adoption of new methodologies for curve-fitting that are used in other fields of environmental science.

The Water Framework Directive (WFD) has triggered much research on non-linear relationships between physico-chemical stressors and ecological responses during the last decade. For example, thresholds in stressor-response relationships are particularly relevant for assessment of ecological status of water bodies. Basic statistical methods such as linear regression or ANOVA are not suitable for estimating such relationships. The response may also be more complicated than e.g. a simple sigmoid curve, threshold-like or circular regressions have been used for exploring the shape of the response curve without a priori assumptions. Moreover, we might be interested in estimating an extreme part of the response rather than the average. In this presentation, I give examples of ecological responses to eutrophication and acidification stress in lakes. The ecological responses are univariate indices representing community composition of phytoplankton, macrophytes, macroinvertebrates and fish. Building upon basic linear regression methods used for studying ecological indicator species, we propose a new approach which enables more flexible curve-fitting: (1) Using a non-linear link to the response variable (generalised linear model); (2) Using a piece-wise linear function for the stressor variable (generalised additive model); (3) Analysing a quantile of the response variable (instead of the average (e.g. 90%); quantile regression). All analyses are carried out in the free statistical programming software R.

TO 335
An interfaced R-package to fit SSDs
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Species Sensitivity Distributions (SSDs) rely on the assumption that aquatic species of a community or assemblage differ in their sensitivity to a hazardous chemical. Toxicity values are used as indicators of the sensitivity and are assumed to follow a theoretical distribution accounting for the inter-species variability. A Hazardous Concentration (HC) is then derived using a low-order percent of the SSD fitted to a limited (and often small) set of toxicity values, the latter being assumed to be a representative sample of species sensitivity among the community to be protected.

The SSD approach is an element of most regulatory guidance documents for environmental risk assessment (e.g. ANZECC, ECHA, USEPA). Yet, the approach to be used is still debated, including: (1) whether parametric or non-parametric methods should be preferred - the applicability of the latter being limited by the small size of toxicity data sets available and (2) in the parametric cases: (2a) the choice of the distribution shape - this is often decided on the basis of mathematical properties, especially on the existence of analytical results, and (2b) the choice of the method for estimating HCs and their confidence interval.

Several softwares have been provided through environmental protection agencies for estimating HCs and their companion uncertainty, such as BURRIZLO in Australia and New Zealand, or ETDX in The Netherlands or Web-ICE in the U.S. Those three softwares have been developed as turn-off-key tools for the application of regional regulatory recommendations and rely on different choices regarding the three points aforementioned. Besides, it appears that none of these programs allows censored data to be accounted for. For users interested in examining the impact of the methodological choices made in existing softwares (and in the corresponding regional guidance documents), we are currently developing a new and open source tool enabling the comparison of several distributions and also the use of both pointwise and interval-censored data (or a mix of both).

TO 336
A probabilistic model for species sensitivity distributions taking into account inherent uncertainty and variability of effects to estimate environmental risk
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Empa, St. Gallen, Switzerland

TU 337
Toxicokinetics-Toxicodynamics survival model, from theory to practice
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Toxicokinetics-Toxicodynamics (TKTD) models simulate the time course of processes leading to toxic effects on organisms. Even for an apparently simple endpoint such as survival, a large number of TKTD models exist. They have been reviewed by Jager et al (2011) who proposed a unified theory (GUTS) for TKTD threshold models and the use of a likelihood approach for estimating model parameters. Here, we focus on a TKTD threshold model in the GUTS framework called DEBox (Dynamic Energy Budgets in Toxicology) survival model. This model assumes an effect above a threshold concentration. We examine a simple question: is it possible to estimate parameters of this model by maximum likelihood approach from standard survival data sets?

We analyzed survival data sets of the freshwater invertebrate Daphnia magna exposed during at least 21 days to nine different contaminants. We tried to fit 4 nested DEBox survival models to each data set by maximizing the log-likelihood function to estimate model parameters.

We illustrate two structural problems linked to the model itself or to the likelihood function. (1) We cannot simplify the model by fixing the natural mortality to 0 even if data did not show natural mortality and (2) because of discontinuities in the log-likelihood cross sections when the threshold parameters is equal to one of the tested concentrations, there are many local minima. This is due to the structure of the threshold model and especially to its non-derivability at the threshold value. We will investigate various solutions to this issue, including adaptation of optimization algorithm and modification of models with fully derivable functions.

TO 338
The IBR revisited: optimization to avoid misuse
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Species Sensitivity Distributions (SSDs) rely on the assumption that aquatic species of a community or assemblage differ in their sensitivity to a hazardous chemical. Toxicity values are used as indicators of the sensitivity and are assumed to follow a theoretical distribution accounting for the inter-species variability. A Hazardous Concentration (HC) is then derived using a low-order percent of the SSD fitted to a limited (and often small) set of toxicity values, the latter being assumed to be a representative sample of species sensitivity among the community to be protected.

The IBR is the sum of the area defined by the k biomarkers arranged in a radar diagram. However, in their initial publication, the authors provide two calculation methods: the first one is a complicated formula that works whenever the number of biomarker is, while the second one is a simplified formula that works only when 4 biomarkers are used. The attractiveness for simplicity led to frequent misuse of the IBR (40% of the 38 publications using it). Moreover, the final outcome of the calculation process highly depends on the sequential organisation of the biomarkers. We aim to propose a new procedure to resolve these main problems in the IBR application.

We first go back to trigonometry basics to provide a new formula for the IBR, that is far more simple than the original one. Then we wrote a procedure that creates all the possible circular permutations of k biomarkers. It results on a (k!-1) matrix of IBR values that allows to calculate the mean IBR for a site and to prioritize IBR values among sites in a more confident way.

A case study using this method is presented, based on the results of a survey of 8 sites presenting contrasted levels of sediment contamination. Several tools have been proposed to integrate these responses in a single and simple measure. Among them, the Integrated Biomarker Response (IBR) is a promising method that provides both a graphical synthesis of the different biomarker responses and a numeric value that is still debated, including: (1) whether parametric or non-parametric methods should be preferred - the applicability of the latter being limited by the small size of toxicity data sets available and (2) in the parametric cases: (2a) the choice of the distribution shape - this is often decided on the basis of mathematical properties, especially on the existence of analytical results, and (2b) the choice of the method for estimating HCs and their confidence interval.

Several softwares have been provided through environmental protection agencies for estimating HCs and their companion uncertainty, such as BURRIZLO in Australia and New Zealand, or ETDX in The Netherlands or Web-ICE in the U.S. Those three softwares have been developed as turn-off-key tools for the application of regional regulatory recommendations and rely on different choices regarding the three points aforementioned. Besides, it appears that none of these programs allows censored data to be accounted for. For users interested in examining the impact of the methodological choices made in existing softwares (and in the corresponding regional guidance documents), we are currently developing a new and open source tool enabling the comparison of several distributions and also the use of both pointwise and interval-censored data (or a mix of both).

TU 339
Statistics Service - a new tool for efficient data analysis
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In this talk we present a newly built statistics tool which allows a highly convenient and efficient data analysis. The tool combines Excel (a Microsoft Office product) and R (a cost-free available statistics programming language) via a specific server.

This Excel-R connection significantly broadens the spectrum of available statistical methods in Excel. Moreover, the user (e.g. lab technician) can easily conduct complex regression, survival, and adoption of new methodologies for curve-fitting that are used in other fields of environmental science.

Environmental Protection agencies have been providing regional regulatory estimations for estimating HCs and their companion uncertainty, such as BURRIZLO in Australia and New Zealand, or ETDX in the Netherlands or Web-ICE in the U.S. Those three softwares have been developed as turn-off-key tools for the application of regional regulatory recommendations and rely on different choices regarding the three points aforementioned. Besides, it appears that none of these programs allows censored data to be accounted for. For users interested in examining the impact of the methodological choices made in existing softwares (and in the corresponding regional guidance documents), we are currently developing a new and open source tool enabling the comparison of several distributions and also the use of both pointwise and interval-censored data (or a mix of both).

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SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
organic contaminations.
Long-term effects of an early exposure to PAHs on zebrafish behavioural responses
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Policymycotic aromatic hydrocarbons (PAH) emission in the environment is constantly increasing with human activity. The goal of this study was to assess long term consequences of fish exposure to PAH on behaviour. Eggs were collected from AB zebrafish strain and were placed at 4 hours post fertilization (hpf) until 96 hpf in a 3 cm diameter Petri dish containing 3 g reference sediment either plain (control) or spiked with a mixture of Benzo[a]pyrene, Pyrene and Phenanthrene at environmental concentrations based on values measured in the Seine Estuaire (France), a highly polluted site. Thereafter, larvae were transferred in clean water and raised until adulthood. Behavioural tests such as locomotion during 24 hrs, sudden dark challenge and T-maze exploration were performed at adults’ stage (F0) and sudden dark change on larvae produced by F0 adults. In adults, during night or dark period, contaminated fish were significantly less active than control fish. A similar observation was made for contaminated F0 in F1 maze safe area. Contaminated F1 larvae were more active when light was on than control and less active when the light was off. This study indicates that contamination of zebrafish using environmentally relevant concentration during the very first stage of development with a PAH mixture of 3 molecules induced late behavioural effects measured at the adult stage. Further, effect was transmitted to the next generation and behavioural responses of F1 larvae were different to that of F1 control larvae. Consequences on fish abilities (e.g. predator escape, food search, courtship) will be discussed.

Long-term food-exposure to PCB mixtures induces reproductive and behavioural disruptions in zebrafish
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Although the use of polychlorinated biphenyls (PCBs) has been banned for several decades, they are still present in the environment. Field analyses have established correlations between exposure to PCBs and alterations in fish physiology including reproductive function and behaviour. In the present study, we performed a life-cycle exposure using zebrafish and mixtures representative of some environmental situations in terms of compositions, dosages and containing mainly non dioxin-like congeners. Exposure was performed through diet which is the main contamination route in the wild. We demonstrated a bioaccumulation of PCBs in males and females as well as a maternal transfer to the eggs. Several reproductive traits were altered after exposure to a PCB-contaminated diet, including a reduction in the number of fertilized eggs per spawn as well as an increase in the number of poorly fertilized eggs/spawn. This was related to modifications of ovary histology revealing a decrease of maturing follicles and an increase of atretic follicles in the ovaries of females exposed to PCBs. In addition, several behavioural traits were monitored. Fish exposed to the highest dose (equivalent to that found in the Seine Estuary) displayed an increased swimming activity during the first 24 hours after hatching. Fish exposed to the intermediate dose (equivalent to that found in the Loire Estuary) displayed behavioural disruption analogous to hyperactivity that showed some similarities to a human behavioural deficit syndrome known as attention deficit/hyperactivity disorder (ADHD), which can be observed in humans after exposure to PCBs. These results indicated that exposure to PCB mixtures mimicking some environmental situations, can lead to a dramatic reduction in the number of offspring produced by a female over a lifetime and disrupt behaviour. Potential ecological consequences are discussed with regard to reproduction, fitness and survival.

Indirect estimation of population-level effect of pollutants based on tolerance evolution and fitness cost of tolerance
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Evolution of tolerance to a pollutant chemical in a natural population of organisms gives an evidence of the examined population to have suffered adverse effect due to the chemical because the tolerance evolution of a population is solely brought about by selection pressure that removes sensitive individuals or genotypes from the population. Despite the importance of using the tolerance evolution as a monitoring tool of chemical pollution in the field, such approach is not relevant for a quantitative impact evaluation because observed differences in the tolerance between a contaminated and an uncontaminated site are rarely connected to the ecological risk that burdens the population. Applying the theory of evolutionary ecology and quantitative genetics into the tolerance evolution, we attempted to estimate the selective force that is needed to maintain the observed difference in the tolerance between populations. For this aim, we made a heuristic assumption for the evolutionary analysis of tolerance: the strength of tolerance induced by a genotype to a particular compound is positively associated with fitness cost of the tolerance, and the joint action of selection induced by the cost and selection favoring higher tolerance achieves optimization of the total fitness that takes into account the fitness gain by the tolerance and the fitness cost due to the tolerance. The fitness gain by the tolerance means reduced toxicity of a specific exposure concentration in comparison to sensitive individuals, and can be quantified by the life table toxicity data. As a case study of such an approach, we detected inter-population-differences of the fenvalerate tolerance among natural populations of a water flea (Daphnia galeata) in Kasamigaura Lake (Japan) and an agricultural reservoir near to this lake. We found a statistically significant negative association, among isolate colonies, between tolerance values and intrinsic population growth rates under null exposure. With the fitness optimization, we derived an estimate of stationary exposure level of the chemical (all compounds that exhibited co-tolerance with this chemical) as 0.015 ppb, and concluded that the examined population of this species had been suffering the population-level effect that corresponded to a 4% reduction of the intrinsic population growth rate. The indicated level of the population-level effect may have considerably reduced the probability of persistence of the population.

Triclosan toxicity: a multi-generational and demographic assessment using Daphnia magna
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2University of Saskatchewan, Saskatoon, Canada
3University of Regina, Regina, Canada

Triclosan is a ubiquitous compound in wastewater treatment plant effluents and biosolids and is commonly detected in surface waters throughout North America and Europe. The acute toxicity of triclosan (TCS) toward aquatic organisms has been well studied but information on long-term, low level exposures at environmentally relevant concentrations is lacking. The objective of the present study was to evaluate the toxicity of triclosan in a multi-generational test with Daphnia magna in laboratory exposures. 24-hr old D. magna neonates were exposed to TCS (0.5 to 150 µg/L) in six successive 21-d life cycle tests. Brood randomly collected from 5 replicate beakers within each treatment on day 21 were used to seed the next generation. Survival across the 6 generations exceeded 80% in all treatments across all generations. A slight decline in population levels at the three highest treatments based on the first 3 generations but not the last 3 generations. In this study, effects were only observed at TCS concentrations approximately one order of magnitude greater than those found in surface waters, which indicates that the long-term risks of TCS are likely minimal.

Assessing the chronic aquatic toxicity of phthalate ester plasticizers
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Phthalate esters are a class of chemicals varying greatly in terms of uses, properties and toxicity. C1 to C4 phthalate esters are used in non-vinyl commercial products and pharmaceuticals. C6 to C10 phthalate esters are at the primary endpoint of regulatory concern. The purpose of the primary endpoint study is to assess chronic effects of phthalate esters on aquatic organisms. Studies show that populations of fish and invertebrates may be adversely affected by exposure to C1 to C4 phthalate esters, but are not adversely affected by exposure to C6 or higher phthalate esters. Secondary endpoints, including molecular, biochemical, and/or histological responses to chemical exposure, do not appear to correspond to primary endpoints of survival, growth and development, or reproductive fitness. A previously published risk assessment for C1 to C4 phthalate esters demonstrated low risks in North American and Western European surface waters. Risk assessments conducted by authorities in Europe with DEHP, DINP, and DIDP have concluded no risks to aquatic organisms due to aqueous solubility constraints, low expected surface water concentrations, and metabolic biotransformation capacity. Important chronic aquatic toxicity studies that have included transgenerational exposure have shown that these assessments and are presented here. The data from the present study, support the earlier risk assessment conclusions. The data also provide further support for a narcosis-related aquatic solubility cutoff at approximately C6 and higher phthalate esters. Finally, for the C1 to C4 phthalate esters, the present study shows that secondary endpoints (e.g., molecular, biochemical, and/or histopathology) to date, provide limited benefit in practical ecological risk assessment of phthalate esters to aquatic species.
Effects of land use and pesticide exposure on hatching characteristics of Daphnia magna epiphipphia

S. Navio, A. Waterkeyn, T. Voet, L. Brendonck

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Daphnia is a well established model organism and standard test species in ecotoxicology. D. magna reproduces by cyclical parthenogenesis, where environmental cues associated with unfavourable conditions trigger sexual reproduction. In permanent and temporary standing waters, sexually produced encapsulated dormant eggs (epiphipphia), build the basis for a dormant resting egg bank, which is important for long-term survival of the population. Each year a fraction of the dormant eggs hatch from the sediment. Through this benthic-pelagic coupling, structure and function of resting egg banks have important consequences for ecological and evolutionary dynamics of the active populations and communities. To date however, almost no information is available on the effects of pollution on these dormant egg banks.

In a recent study, we showed that the earthworm species Eisenia fetida, inhabiting an extremely high metal polluted compost heap on a wine farm, did not have elevated copper, was assessed. Subsequently, to evaluate its acclimation to salinity, each clonal lineage was acclimated to 0.1 g of NaCl for two generations and its lethal and sublethal objectives, the lethal and sublethal (growth and reproduction) sensitivity to salinity of six clonal lineages of Daphnia longispina, exhibiting different lethal resistances to populations we aim to aid in developing new bio-monitoring approaches and provide advanced scientific basis for integrative ecological risk assessment methodologies which are essential for effective environmental conservation strategies. The SETAC meeting is an excellent opportunity to share ideas, approaches, methodologies and preliminary results with other researchers working in the field of evolutionary toxicology.

Metal toxicity in the earthworm Eisenia fetida: Is it a case of adaptation or mere physiological acclimation? O.J. Owojori, E. Poua-Otomo, S.A. Reinecke, A.J. Reinecke

ECT Oekotoxikologie GmbH, Florsheim, Germany

Department of Plant and Zoology, Stellenbosch University, Stellenbosch, South Africa

A recent study showed that the earthworm species Eisenia fetida inhabiting an extremely high metal polluted compost heap on a wine farm, did not have elevated body loads of the metals but exhibited genotoxic tolerance when exposed to Cd in the laboratory (Poua Otomo and Reinecke, 2010). To unravel the mechanism behind the surprisingly low body metal burdens on one hand and genotoxic tolerance on the other hand, we investigated the estimated bioavailability of these metals (Cu, Zn, Pb and Cd) using sequential extraction methods with CaCl2 and di-ethylene-triamine-pentaacetic acid (DTPA) and allozyme polymorphism in this field population, a laboratory reproduces by cyclical parthenogenesis and long-term Cu and Zn levels. Additional full dose-response experiments were carried out with the most and least sensitive populations. Our results indicate that several fitness traits have the potential to respond to natural selection and genetically adapt, but that this depends strongly on the population and the exposure treatment considered. In long-term multi-generational exposures this may result in shifts in genotype frequencies and reduction of genetic diversity.

TU 349
Evolutionary consequences of the loss of genetic diversity in C. riparius populations - Introducing the MIDGE project


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Chromosomally determined frame shift mutations have been shown to decrease genetic variation in populations of several species alongside with adverse effects on the physiology of organisms. This loss of genetic diversity may reduce the potential of populations to adapt to changing environments.

Ecotoxicological studies need to consider not only short term effects of pollution, such as changes in life history traits of organisms, but also its long term effects, such as genetic loss. This genetic loss can lead to the loss of evolutionary potential and thus it is also of crucial importance to assess the evolutionary consequences of changes in genetic variability.

There is an urge for evolutionary toxicology studies because only an integrated approach linking contamination, genetic variability, life-history responses and fitness costs associated with adaptation can lead to effective prediction of the genetic impacts on biodiversity.

In the MIDGE project aims to study the Microevolutioanry Dynamics and Genetic Erosion in pollution-affected Chironomus populations. More specifically we are investigating the genetic variability of C. riparius populations from unpolluted and contaminated sites using mitochondrial sequence variation and nuclear microsatellite analyses and we intend to address three main questions:

- Does chronic pollution affect genetic variability of C. riparius populations in the field?
- Are C. riparius populations in contaminated areas adapted to pollution exposure?
- What are the consequences of this altered genetic variability due to pollution in terms of fitness costs?

Measures of genetic erosion have recently been proposed as the ultimate biomarker of effect and by focusing on effects of contaminants on genetic variability in natural populations we aim to aid in developing new bio-monitoring approaches and provide advanced scientific basis for integrative ecological risk assessment methodologies which are essential for effective environmental conservation strategies. The SETAC meeting is an excellent opportunity to share ideas, approaches, methodologies and preliminary results with other researchers working in the field of evolutionary toxicology.

TU 349

Association between increased resistance to copper and salinity in Daphnia longispina clonal lineages under short- and long-term multi generational exposures

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Presently, many coastal freshwater ecosystems are already exposed to chemical contaminants. Depending on the period and intensity of such exposure, the selective pressure exerted by chemical contamination could have caused selection on the most sensitive genotypes from exposed population, causing its’ genetic erosion. Whether such endured populations will be capable of coping with future environmental perturbations, namely those associated with the predicted climate changes such as the gradual increase in temperature in coastal lagoons, will largely depend on the association between the resistance to chemical it was exposed to and the future environmental stressor. Accordingly, this work aimed at assessing if an increased resistance to copper is associated with an increased sensitivity to salinity; or at least if some of the genotypes most resistant to copper are the most sensitive to salinity, both under (i) short-term exposures and under (ii) long-term generational exposures. To attain these objectives, organisms from different populations of Daphnia longispina, exhibiting different lethal resistances to copper, was assessed. Subsequently, to evaluate its acclimation to salinity, each clonal lineage was acclimated to 0.1 g of NaCl for two generations and its lethal and sublethal sensitivity to salinity was re-assessed. The observed results, revealed significant associations between an increased resistance to copper and the lethal or sublethal sensitivity to salinity of organisms from contaminated populations. More specifically we are investigating the genetic variability of C. riparius populations from unpolluted and contaminated sites using mitochondrial sequence variation and nuclear microsatellite analyses and we intend to address three main questions:

- Does chronic pollution affect genetic variability of C. riparius populations in the field?
- Are C. riparius populations in contaminated areas adapted to pollution exposure?
- What are the consequences of this altered genetic variability due to pollution in terms of fitness costs?

Measures of genetic erosion have recently been proposed as the ultimate biomarker of effect and by focusing on effects of contaminants on genetic variability in natural populations we aim to aid in developing new bio-monitoring approaches and provide advanced scientific basis for integrative ecological risk assessment methodologies which are essential for effective environmental conservation strategies. The SETAC meeting is an excellent opportunity to share ideas, approaches, methodologies and preliminary results with other researchers working in the field of evolutionary toxicology.

TU 350

Metal tolerance in the earthworm Eisenia fetida: Is it a case of adaptation or mere physiological acclimation?

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A recent study showed that the earthworm species Eisenia fetida inhabiting an extremely high metal polluted compost heap on a wine farm, did not have elevated body loads of the metals but exhibited genotoxic tolerance when exposed to Cd in the laboratory (Poua Otomo and Reinecke, 2010). To unravel the mechanism behind the surprisingly low body metal burdens on one hand and genotoxic tolerance on the other hand, we investigated the estimated bioavailability of these metals (Cu, Zn, Pb and Cd) using sequential extraction methods with CaCl2 and di-ethylene-triamine-pentaacetic acid (DTPA) and allozyme polymorphism in this field population, a laboratory control as well as a long-term Cd exposed population. The amounts of mobile (extracted with CaCl2) and mobilisable (extracted with DTPA) metals in relation to the total metals (Cu, Zn, Pb and Cd) using sequential extraction methods with CaCl2 and di-ethylene-triamine-pentaacetic acid (DTPA) and allozyme polymorphism in this field population, a laboratory control as well as a long-term Cd exposed population. The amounts of mobile (extracted with CaCl2) and mobilisable (extracted with DTPA) metals in relation to the total metals (Cu, Zn, Pb and Cd) using sequential extraction methods with CaCl2 and di-ethylene-triamine-pentaacetic acid (DTPA) and allozyme polymorphism in this field population, a laboratory control as well as a long-term Cd exposed population. The amounts of mobile (extracted with CaCl2) and mobilisable (extracted with DTPA) metals in relation to the total metals (Cu, Zn, Pb and Cd) using sequential extraction methods with CaCl2 and di-ethylene-triamine-pentaacetic acid (DTPA) and allozyme polymorphism in this field population, a laboratory control as well as a long-term Cd exposed population. The amounts of mobile (extracted with CaCl2) and mobilisable (extracted with DTPA) metals in relation to the total metals (Cu, Zn, Pb and Cd) using sequential extraction methods with CaCl2 and di-ethylene-triamine-pentaacetic acid (DTPA) and allozyme polymorphism in this field population, a laboratory control as well as a long-term Cd exposed population. The amounts of mobile (extracted with CaCl2) and mobilisable (extracted with DTPA) metals in relation to the total metals (Cu, Zn, Pb and Cd) using sequential extraction methods with CaCl2 and di-ethylene-triamine-pentaacetic acid (DTPA) and allozyme polymorphism in this field population, a laboratory control as well as a long-term Cd exposed population. The amounts of mobile (extracted with CaCl2) and mobilisable (extracted with DTPA) metals in relation to the total metals (Cu, Zn, Pb and Cd) using sequential extraction methods with CaCl2 and di-ethylene-triamine-pentaacetic acid (DTPA) and allozyme polymorphism in this field population, a laboratory control as well as a long-term Cd exposed population. The amounts of mobile (extracted with CaCl2) and mobilisable (extracted with DTPA) metals in relation to the total metal
webs linking primary producers to consumers. The negative effects on Daphnia are well understood; toxic cyanobacteria affect growth, survival and reproduction. However, one possibility to ameliorate toxic effects is to biotransform MC by conjugation to glutathione via glutathione S transferase, thereby reducing toxicity and aiding excretion. This process is thought to underlie the ability to withstand MC in Daphnia and could explain the enhanced tolerance found in the offspring of Daphnia exposed to live cyanobacteria.

In this study, multigeneration exposures were conducted, we exposed the parental generation to MC-LR for 1 or 7 days and determined the enzyme mediated tolerance to MC in their offspring, a) by assessing the acute effect of MC-LR on biotransformation, antioxidant and energy metabolism enzyme activities, and b) through 21 day chronic toxicity, via growth and membrane integrity assay. Seven day exposure of the parental generation to MC-LR induces higher activity of glutathione S transferase and malate dehydrogenase in the offspring and enables it to increment the catalase activity when challenged with the toxin. In offspring from the 1 day exposed parental generation those effects were less pronounced or not visible. Offspring from 7 day exposed females suffered high mortality and death of eggs and larvae when exposed to MC-LR whereas the offspring from 7 day exposed mothers show higher survival. Higher survival of the offspring is correlated with the elevated activity of glutathione S transferase, malate dehydrogenase and catalase, suggesting maternal transfer of activation factors.

These parental transgenerational or maternal effects provoked by a natural toxin in a very important freshwater herbivore may explain the observed enhancement of tolerance over generations through a biochemical perspective.

**TU 352**

**Is there a functional role of DNA methylation in the stress response?**

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DNA methylation, the addition of methyl groups on the 5’ position of cytosines in the DNA plays an important role in the regulation of gene expression of eukaryotic cells. Computational studies have been performed in relationships of DNA methylation in invertebrates has only recently received attention. In particular for Daphnia magna, a key test organism in aquatic toxicology and an important species in many aquatic ecosystems, knowledge on DNA methylation is very limited. Within our long-term aim of unravelling the potential functional role of DNA methylation in the stress response of D. magna, this study aimed at sequencing the entire cytosine methylome.

For the comprehensive profiling of DNA methylation a methylated binding domain (MBD) protein based sample preparation technique was used. Since this technique is very specific and works well with fragments densely methylated, it was decided to use also a less specific technique based on DNA digestion with a novel restriction enzyme. This endonuclease recognizes the CNNR (R=G/A) methylated sites and cleaves DNA at fixed distances generating DNA fragments easily identified on gel.

Bioinformatics analysis (i.e. mapping with the most recent D. magna genome assembly) was performed in order to generate the methylome map. Ongoing bioinformatic analyses is now focused on determining structural, functional or evolutionary relationships among the methylated sequences and to determine if methylated sequences occur in genes or pathways that may be relevant for dealing with chemical and ecological stress.

**TU 353**

**Effects of benzo(a)pyrene and vinclozolin on freshwater snails: I) epigenetic and genetic responses of adults and II) offspring embryo toxicity**

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Vinclozolin causes transgenerational effects by inducing DNA methylation changes in exposed rats. Benzo(a)pyrene is a clastogen used as a reference chemical for the in vitro mammalian cell micronucleus test. Taking into account that epigenetic and genetic profiles could be altered by these pollutants we studied molecular and cellular responses after adult male exposure to fresh water snails (Physa acuta). Additionally, the embryo toxicity in the following generation (F1) after paternal exposure was evaluated. Mature snails exposed for 46 days and deposited egg masses were used for determining parental endpoints (F0) and transgenerational effects, respectively. DNA extracted from mature snails were analysed to evaluate differences in DNA methylation patterns in order to analyze methylation-sensitive sites we used PCR technique with arbitrary primers (AP-PCR) using HpaII and MspI digested samples. HpaII and MspI are isoschizomers that recognize the tetranucleotide sequence 5’-CCGG-3’ but that show differential cleavage sensitivity to cytosine methylation. HpaII cannot cleave it one or both cytosines are fully methylated in both strands), whereas MspI cleaves C5mCGG but not 5mCCGG sequences. Gel electrophoresis of PCR products and comparative analysis between HpaII and MspI patterns allowed the study of CCGG regions in the genome that are sensitive to methylation and the effect of these pollutants on these sites. Genotoxicity was measured by the micronucleus test in cells isolated from whole animals at the end of the experiment. Finally, survival and development of offspring was measured. All masses were recovered and the teratogenic effects of both non-exposed and exposed single egg capsules were measured in multiwell plates. Parental responses at cellular and molecular levels were compared with F1 responses using an ecological relevant endpoint (embryo toxicity). The aim of this work was to establish potential linkages between mechanisms of action and long-term responses at higher levels of biological organisation, which could ultimately have consequences on population dynamics. This study was supported by the Spanish research project CTM2008-03492.

**TU 354**

**Late-life effects of early-life mitochondrial DNA damage**

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For long-lived species, exposure to environmental contaminants can have delayed effects later in life. DNA methylation is a mechanism that can be acted on by environmental chemicals and can lead to delayed responses. In the past, we have shown that exposure to PAHs, PCP, metals, and plastics during early life can lead to delayed effects on metabolic enzymes in Daphnia magna. These results prove transgenerational or maternal effects provoked by a natural toxin in a very important freshwater herbivore which may explain the observed acquisition of enhanced tolerance over generations through a biochemical perspective.

**TU 355**

**Is trans-generation PCB transfer a key process for parental detoxification and adaptation in amphipods?**

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PAHs, and metals) of sediment and organisms to gain information about potential cause-effect relationships. The integrated analysis of the data showed that the nature of contaminant, the age of the exposed generation and the exposure duration and intensity can have a considerable impact on the interactions identified between the contaminants and the ecosystem. However, the mechanisms through which these interactions occur are not yet well understood. A method which assesses soil health by analyzing the functional stability of soil enzymes, fission and autofyisis. Mutations in mitochondrial fusion and autophagy genes exacerbate the larval arrest, suggesting a potent gene-environment interaction in which the effects of mtDNA damage caused by environmental agents are exacerbated by decreased mitochondrial fusion and autophagy.

**TU 356**

**The enzymatic functional activity of zinc-tolerant microbial communities: a new approach to detect terrestrial ecotoxicity of metals**

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The increased levels of metals exposed to metals resistant microorganism can result in the depletion of their biodegradative chemicals and become metal-tolerant. This tolerance could increase the vulnerability of the microbial community to additional soil disturbances and then decrease the soil microbial functional stability. The functional stability is defined as the ecosystem capacity to recover its biodegradative functions after a disturbance. This concept is more and more studied because this approach could detect ecotoxic effects which would not have been found by common microbial bioindicators. A method which assesses soil health by analyzing the functional stability of soil enzymes has been set during the last years and has developed a stability indicator named the Relative Soil Stability Index (RSSI). These indexes were calculated to assess the enzymatic functional stability of 9 contaminated metal contaminated sites after a heat degradation (desiccation at 60°C for 24h). Four enzymes were analyzed: arylosulfatase, acid phosphatase, protease and urease. The RSSI of arylosulfatase and protease were linearly correlated to the labile zinc concentration in the soils (arylosulfatase : R² = 0.63 for n=6, t = 0.95), while the enzymatic activity of these enzymes was not correlated to the labile metal fraction. These results suggest that the sulfur and nitrogen cycles would be unstable in contaminated zinc contaminated soils. This potential ecotoxic effect would not have been detected if only the enzymatic activity has been analyzed.
TU 358

Does genetic adaptation matters? An hypothesis tested using life-history consequences of adaptation and acclimation to copper of Daphnia longispina
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The present study was conducted to test the hypothesis that genetically adapted clones of Daphnia longispina showed a higher fitness performance under copper exposure than acclimated clones. Genetically tolerant and sensitive clones were selected from a reference and a historically exposed populations to an acid mine drainage from an abandoned pyrite mine. Four sensitive and resistant clones from reference and impacted populations were expose to a range of copper exposure levels and their life history responses in terms of survival, reproduction and population growth rates compared. In another experiment the most sensitive clone was exposed during four generations to the same range of copper concentrations and its life-history performance compared. The genetic differences between the resistant and sensitive clones were that in the polluted environment the resistant clone produced three more offspring per day and had population growth rate 45% higher. In the unpolluted environment, however, individuals from the resistant clone had the lowest reproduction rate. After four generations acclimated individuals from the sensitive clone reproduce earlier, had greater clutches at first reproduction and higher population growth rates than none acclimated ones but apparently there were no fitness costs. Therefore, our results showed that life history changes due to adaptation and acclimatization were compatible under the polluted environment but not under a clean environment. This results support the view that genetic adaptation is ecologically costly but acclimatization not.

TU 359

Evaluating effects of pollution on Caenorhabditis elegans’ population dynamic through a bio-energetic approach
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The assessment of toxic effects at biologically and ecologically relevant scales is an important issue in ecosystem protection. Mathematical models exist to predict effects of pollutant on population dynamics from individual data. Nevertheless there are only a few datasets and models that account for adaptive phenomena which may appear in a stressed population. The selection pressure exerted by a pollutant is known to amplify the phenomenon of natural selection. It is thus essential to understand and quantify the adaptive dynamics governing populations under stress in order to assess ecological risk.
Regarding this background, we adapted a bioenergetic model to study adaptive phenomena in Caenorhabditis elegans population dynamic exposed to a heavy radiotoxic metal (uranium). The Dynamic Energy Budget (DEB) (Kooijman, 2010) bioenergetic approach highlights the distribution of energy fluxes between processes such as growth, reproduction, maturation and maintenance. It is a relevant basis to understand and model the links between assimilation disruptions, growth and reproduction fluctuations in organisms exposed to anthropogenic stressors (e.g. pollutants, global change) and to assess potential consequences on population over many generations.
We therefore studied the responses of C. elegans exposed to six experimental concentration of uranium over several generations. The individual traits followed were number of colony forming units per milliliter values (410 ± 26 cfu mL⁻¹ and 383 ± 91 cfu mL⁻¹ respectively). On the other hand Aveiro (0.26 ± 0.09%) and Setúbal (45.5%) while Setúbal had the lowest (2.56%) . Regarding 1mM and 3mM concentrations, Peniche had the highest percentage rate (8.2% and 7.7% respectively) as well the highest number of colony forming units per milliliter values (410 ± 26 cfu mL⁻¹ and 383 ± 91 cfu mL⁻¹ respectively). We have characterized these isolates in what concerns growth rate in the presence of increasing concentrations of TBT and later identified them by 16S rRNA gene sequencing. Bacterial diversity was also assessed on the same range of copper concentrations and its life-history performance compared. The genetic differences between the resistant and sensitive clones were that in the polluted environment the resistant clone produced three more offspring per day and had population growth rate 45% higher. In the unpolluted environment, however, individuals from the resistant clone had the lowest reproduction rate. After four generations acclimated individuals from the sensitive clone reproduce earlier, had greater clutches at first reproduction and higher population growth rates than none acclimated ones but apparently there were no fitness costs. Therefore, our results showed that life history changes due to adaptation and acclimatization were compatible under the polluted environment but not under a clean environment. This results support the view that genetic adaptation is ecologically costly but acclimatization not.

TU ET11 - Marine environmental chemistry and ecotoxicology
TU 361

Biotechnological potential of impacted scenarios for the restoration of TBT contaminated environments
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This study was conducted to test the hypothesis that genetically adapted clones of Daphnia longispina showed a higher fitness performance under copper exposure than acclimated clones. Genetically tolerant and sensitive clones were selected from a reference and a historically exposed populations to an acid mine drainage from an abandoned pyrite mine. Four sensitive and resistant clones from reference and impacted populations were expose to a range of copper exposure levels and their life history responses in terms of survival, reproduction and population growth rates compared. In another experiment the most sensitive clone was exposed during four generations to the same range of copper concentrations and its life-history performance compared. The genetic differences between the resistant and sensitive clones were that in the polluted environment the resistant clone produced three more offspring per day and had population growth rate 45% higher. In the unpolluted environment, however, individuals from the resistant clone had the lowest reproduction rate. After four generations acclimated individuals from the sensitive clone reproduce earlier, had greater clutches at first reproduction and higher population growth rates than none acclimated ones but apparently there were no fitness costs. Therefore, our results showed that life history changes due to adaptation and acclimatization were compatible under the polluted environment but not under a clean environment. This results support the view that genetic adaptation is ecologically costly but acclimatization not.

TU 362

Bacterial diversity on Portuguese ports: TBT degrading bacteria
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This study was conducted to test the hypothesis that genetically adapted clones of Daphnia longispina showed a higher fitness performance under copper exposure than acclimated clones. Genetically tolerant and sensitive clones were selected from a reference and a historically exposed populations to an acid mine drainage from an abandoned pyrite mine. Four sensitive and resistant clones from reference and impacted populations were expose to a range of copper exposure levels and their life history responses in terms of survival, reproduction and population growth rates compared. In another experiment the most sensitive clone was exposed during four generations to the same range of copper concentrations and its life-history performance compared. The genetic differences between the resistant and sensitive clones were that in the polluted environment the resistant clone produced three more offspring per day and had population growth rate 45% higher. In the unpolluted environment, however, individuals from the resistant clone had the lowest reproduction rate. After four generations acclimated individuals from the sensitive clone reproduce earlier, had greater clutches at first reproduction and higher population growth rates than none acclimated ones but apparently there were no fitness costs. Therefore, our results showed that life history changes due to adaptation and acclimatization were compatible under the polluted environment but not under a clean environment. This results support the view that genetic adaptation is ecologically costly but acclimatization not.

TU 363

Biotechnological potential of impacted scenarios for the restoration of TBT contaminated environments
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The assessment of toxic effects at biologically and ecologically relevant scales is an important issue in ecosystem protection. Mathematical models exist to predict effects of pollutant on population dynamics from individual data. Nevertheless there are only a few datasets and models that account for adaptive phenomena which may appear in a stressed population. The selection pressure exerted by a pollutant is known to amplify the phenomenon of natural selection. It is thus essential to understand and quantify the adaptive dynamics governing populations under stress in order to assess ecological risk.
Regarding this background, we adapted a bioenergetic model to study adaptive phenomena in Caenorhabditis elegans population dynamic exposed to a heavy radiotoxic metal (uranium). The Dynamic Energy Budget (DEB) (Kooijman, 2010) bioenergetic approach highlights the distribution of energy fluxes between processes such as growth, reproduction, maturation and maintenance. It is a relevant basis to understand and model the links between assimilation disruptions, growth and reproduction fluctuations in organisms exposed to anthropogenic stressors (e.g. pollutants, global change) and to assess potential consequences on population over many generations.
We therefore studied the responses of C. elegans exposed to six experimental concentration of uranium over several generations. The individual traits followed were number of colony forming units per milliliter values (410 ± 26 cfu mL⁻¹ and 383 ± 91 cfu mL⁻¹ respectively). On the other hand Aveiro (0.26 ± 0.09%) and Setúbal (45.5%) while Setúbal had the lowest (2.56%) . Regarding 1mM and 3mM concentrations, Peniche had the highest percentage rate (8.2% and 7.7% respectively) as well the highest number of colony forming units per milliliter values (410 ± 26 cfu mL⁻¹ and 383 ± 91 cfu mL⁻¹ respectively). We have characterized these isolates in what concerns growth rate in the presence of increasing concentrations of TBT and later identified them by 16S rRNA gene sequencing. Bacterial diversity was also assessed on the same range of copper concentrations and its life-history performance compared. The genetic differences between the resistant and sensitive clones were that in the polluted environment the resistant clone produced three more offspring per day and had population growth rate 45% higher. In the unpolluted environment, however, individuals from the resistant clone had the lowest reproduction rate. After four generations acclimated individuals from the sensitive clone reproduce earlier, had greater clutches at first reproduction and higher population growth rates than none acclimated ones but apparently there were no fitness costs. Therefore, our results showed that life history changes due to adaptation and acclimatization were compatible under the polluted environment but not under a clean environment. This results support the view that genetic adaptation is ecologically costly but acclimatization not.

TU 364

Are TBT sources migrating from harbors to marinas in Latin America?
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Tributyltin (TBT) is a very toxic compound that was used as antifouling paints biocide during four decades. Due its environmental toxicity the International Maritime Organization banned TBT based antifouling paints in September 2008. Thereafter, TBT environmental concentrations as well as imposex levels (the most widely used TBT biomarker) declined in several coastal areas worldwide. However, in Latin America some recent observations have shown high TBT and imposex levels in areas close to harbors, despite the general reducing pattern in many areas. The present study reported imposex parameters (imposex %, VDSI and RPl) in gastropods from Venezuela and Brazil in harbor areas and marinas. In Venezuela, samples with 30 adults of Purpura patula were collected in 23 sites (2 located inside marinas). In Brazil, the sampling
collection has been performed for some years at Angra dos Reis (10 out of 33 stations were close to marinas) and Arraial do Cabo (2 out of 10 stations were close to marinas) in Rio de Janeiro state. These studies used 30 adults of Stramonita haemastoma per site (where found). The results for Venezuela showed that the VDSI ranged from 0.22 to 1.86 in areas under the influence of harbors, whereas VDSI values were 3.77 and 4.56 in the marinas. Similarly, RPSI ranged from 0.07 to 5.58 in the harbor areas and were 9.36 and 54.56 to the marinas. In this case, the main source of organotins pollution is clearly the marinas. In the Angra dos Reis study, imposex was detected in 100% of females close to the marinas, and in 5 out of 10 stations the animals previously inhabiting the rocky shores were no longer found. In Arraial do Cabo, two stations nearby a single marina showed an imposex incidence of 4% and 0 in 2001, increasing to 83% and 47% in 2008. In this region, another marina located near a small harbor showed imposex incidence of 0.00% whereas no gastropods were found during 2008 sampling. Thus, although the international restrictions on TBT use are apparently reducing the impacts from harbors, these three independent studies at Venezuela and Brazil (Angra dos Reis and Arraial do Cabo) clearly pointed out that marinas are becoming an important source of TBT pollution. Based on that, a similar pattern is expected to occur in other Latin America coastal areas.

TU 364
Imposex and organotin (OT) levels in Nucella lapillus along the Portuguese coast: a re-survey in 2011
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The use of tributyltin based antifouling paints (TBT-based AIP) is globally forbidden since the 17th September 2008 by the ‘International Convention on the Control of Harmful Antifouling Systems on Ships’ (ACSF Convention). It is therefore important to ensure the continuous monitoring of organotins (OT) in the environment and the recovery of marine ecosystems to evaluate the effectiveness of legislation in reducing TBT pollution. The current work assessed imposex and OT tissue contamination in N. lapillus at 15 stations along the Portuguese coastline in 2011. The vas deferens sequence index (VDSI), the female penis length index (FPLI), the relative penis size index (RPSI), the percentage of imposex affected females (%I) and the percentage of sterile females (%S) were determined to assess imposex levels. Additionally, monobutyltin (MBT), dibutyltin (DBT), TBT, diphenyltin (TPT), triphenyltin (TPT), monoctyltin (MOT) and dioctyltin (DOT) were quantified in the soft tissues of whole females by GC-MS. The OSPAR Ecological Quality Objective (ECQO) set for this species (VDSI < 2) was achieved in 94% of the sampled sites. All surveyed populations presented VDSI values that fall into OSPAR class B (0.3 < VDSI < 2) with one single exception in the South western coast - Zambujeira do Mar (site 15) - also being the only site where sterile females were still recorded. OT concentrations in N. lapillus soft tissues varied between <0.2 ng Sn/g and 24 ng Sn/g dry weight (dw) for MBT, 2.0 and 26 ng Sn/g dw for DBT, 2.1 and 30 ng Sn/g dw for TPT and <0.2 ng Sn/g basa 1.4 ng Sn/g dw for TPT, DOT, MOT and DOT concentrations were all below the detection limit. TBT recent inputs were estimated to occur at four sites by the butyltin degradation index (DBI = [MBT]+[DBT]/[TBT]). The temporal trend of N. lapillus imposex was analysed for the period 2000-2011 in order to evaluate the trend of TBT pollution levels in the Portuguese coast.

TU 365
TBT from anti-fouling paints in the environment - an overview of the situation today
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1Stockholm University, Stockholm, Sweden
2The Baltic Sea is a sensitive brackish water environment where most organisms live at the edge of their distribution area. Pollutants thus mean an extra stress to these organisms. Traditionally anti-fouling paints are based on leakage of biocides, which prevents the attachments of fouling organisms on underwater structures such as a boat hull. The leakage is caused by that the biocides are persistent and that they also affect non-target organisms. The environmental authorities have with time implemented more strict regulations, especially for the paints that may be used on the east coast of Sweden. The use of tin-organic substances were prohibited in 1989 for all boats less than 25 m and no paints based on copper leakage were approved from 2001 and up today no copper paints are on the market for use on the east coast. In spite of these restrictions much imposing treatment from anti-fouling paints continues along both natural harbours and marinas along both the Bohai and the east coast of Sweden. Similarly high concentrations are found in harbours for ships. Our investigations show that the highest concentrations are found in boat yards > uptake areas > marinas > natural harbours. 100 μg TBT/kg DW is considered high in many countries and the highest concentrations in boat yards reached 30 000 μg TBT/kg DW. The reason is under laying paint layer being scrapped off.

TU 366
Comparing anadara trapezia exposure, dose and response to metal contaminated estuarine sediments using laboratory and field exposures and resident molluscs
M. Taylor, W.A. Maher
University of Canterbury, Christchurch, New Zealand

Establishing relationships between metal exposure, internal dose and associated biological effects for organisms is necessary to understand the fate and effects of metals in the environment.

The accumulation and sequestration of biologically available metals by aquatic organisms, particularly bivalve molluscs, has led to their use as biomonitoring of metal contamination in aquatic systems. It is often unclear which pathway(s) of metal bioavailability are contributing to the observed biological effects. This study assesses the relationship between measured external and internal metal exposure of the bivalve Anadara trapezia and the observed biological response, namely imposex, at 16 sites within the Guadiana estuary, Portugal. Imposex occurrence and the genetic changes in Veined Rapa Whelk (Rapana venosa) from Bohai Bay, China

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3National Eain University, Biochomatronic Engineering, Ilan, Taiwan
4Anadara Anadara is common among estuaries in southeastern and northeastern coastal areas of Taiwan. The freshwater clam has a high market value and commercially important to Taiwan’s aquaculture. Previous studies indicate that biomonitoring is the scientific technique for detecting environmental pollution situations for presenting the harmful of toxic chemical in water. The purpose of this study was to synthesize water chemistry-based bioavailability and valve daily rhythm in Corbicula fluminea to design a biomonitoring system for detecting waterborne As. We integrated valve daily rhythm dynamic pattern and water chemistry-based Hill dose-response model to build in a valovoltaic technique programmatic mechanism, offering a rapid and cost-effective dynamic detection system. We validated the simulated dissolved As concentrations based on valve daily rhythm behavior with published experiment data. The results indicated that the As concentration detection threshold of biomonitoring system was set in 0.1 μg/l and the detection times are associated with the exposure concentrations. This study presents a C. fluminea-based biomonitoring system that can particularly provide the real time transmitted information on the waterborne As activity. This parsimonious C. fluminea valve rhythm behavior-based real time biomonitoring system presents a valuable approach to promote the automated biomonitoring and offers early warnings on the potential ecotoxicological risks in regions with elevated As concentration.

TU 369
A comparative in situ study on ecotoxicological effects of pharmaceuticals in Ireland, using marine mussels (Mytilus spp.)
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In recent decades the amount of pharmaceuticals used and released has constantly increased. One of the main sources of this pollution is through the release of wastewater
Chronic exposure of young spat of the Pacific oyster (Crassostrea gigas) to zinc

J. Devois, V. Voiseux1, C. Caplat1, J. Fiévet1

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The marine environmental condition is constantly submitted to many sources of releases from human origins, resulting in low concentrations of contaminants and chronic exposure to these low doses over their entire life. Because biological mechanisms to respond to acute and chronic exposure may be different, the concentration sensitivity is also different. It is thus a challenge that stakeholders in charge in the environment protection regulation can lean on robust scientific basis addressing the consequences of chronic exposure of species to low doses of pollutants. For long life-span species, investigating chronic exposure means long term experiments with low dose challenge with many potential technical biases. A relevant strategy is to use early stages of development. Even if the exposure time is limited compared to the life-span of the organism, if exposure starts at the very beginning, it covers the whole lifetime up to the end of the experiment, which is relevant in terms of chronicity. In addition, early stages of development are often more sensitive to pollutants than adults, so they can be used for ecotoxicology obviously makes sense. Zinc is an essential trace metal in living organisms, widely used by all living species, including many aquatic species. Our study addressed the effects of zinc on Pacific oyster (Crassostrea gigas) spat. Young oysters were exposed to a range of zinc concentrations, added to seawater as ZnCl₂, up to 2 mg L⁻¹, as 1 day post-metaplanctonic and for 10 weeks. They were fed with micro algae at the same cells concentrations. Zinc was measured in seawater and in oysters throughout the experiment. Survival and growth were monitored on large sample populations to deal with individual variability. A dose-response curve analysis allowed deriving the EC₅₀, for growth. In parallel, target genes analysis included heat shock proteins, metallothionein (MT), detoxication machinery, regulation of oxidative stress and cell cycle. Gene transcriptional expression was quantitated by RT real time PC. Significant change was only detected for MT after 1 week exposure to the highest zinc concentration. However growth was affected as early as a few days of exposure to relatively low dose and turned out to be an earlier and more sensitive marker of the effect of zinc on oyster spat.

Assessment of sacrificial anode impact by zinc accumulation in oyster Crassostrea gigas: comparison between a long- and short-term laboratory tests

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In marine environments, sacrificial anodes made of zinc are currently used to mitigate marine corrosion as part of cathodic protection systems of immersed metallic structures. Zinc is an essential metal for living organisms but its potential toxicity when present in excess. The aim of this study was to assess the bioaccumulation and the effects of zinc released using an electrochemical device providing controlled zinc concentrations from sacrificial anode degradation to mimic the in situ conditions. The work was carried out on oyster Crassostrea gigas by performing two in vivo tests. The first test was conducted over a period of 10 weeks at a concentration of 0.33 mg Zn L⁻¹ to simulate short-term exposure and, a second one lasted for 168-hours at a concentration of 2 mg Zn L⁻¹ to simulate long-term exposures. Following exposure, zinc concentrations were measured in oyster gills, digestive gland and soft tissues and, endpoints such as mortality, immune parameters and mRNA expression of genes associated with oxidative stress and cell death were analysed. At the end of both experiments, no oyster mortality rate was recorded, and the bioconcentration factor (BCF) exhibited values up to 405 in the digestive gland. In contrast, the results obtained though chronic exposure showed a higher BCF (13,397), whereas no mortality was detected. Moreover, zinc was able to modulate physiological stress related to reproduction and xenobiotic stress. In this context, terrestrial inputs including pesticides could be involved in oyster mortality events, not as a single causative agent but as additional stressors. The study was carried out in the frame of the European Project Chronoex. The aim of this project was to evaluate the impact of different contaminants (including pesticides) used in the region surrounding the English Channel on the early stages of Crassostrea gigas. Experiments were conducted on oyster spat, a model sensitive to environmental contamination by xenobiotics and used as a keystone species to test different environmental conditions. The effects of these substances and copper sulphate were studied in D-hinged larvae in order to assess their potential embryotoxicity by comparing the sensitivity of normal larval development related to the kind of abnormality (multi-variated analyses). The effects were also researched in pediveliger larvae ready to metamorphosis (calculations of metamorphosis rates). For metamorphosis test, we optimized Cooch & Bonnar’s protocol because of the difficulty to count moving larvae. For each endpoint (embryo larval development and metamorphosis rates), results of 4 experiments allowed us to determine the EC₅₀ values. As regards embryotoxicity, 2 kinds of Roundup appeared about 10-fold more toxic than glyphosate and AMPA and the toxicity of 2M4C reached 3.5-fold of those active substances. In the metamorphosis assay, a similar result was recorded and, glyphosate, AMPA and mecoprop appeared little toxic (EC₅₀ >100 mg L⁻¹). Finally, the comparison between the 2 larval stages generally showed a greater sensitivity of D larvae (embryotoxicity) compared to pediveliger larvae. For most of the molecules, results showed that the toxicity of Zn and insecticides increased with the larval stage. This is not the case for the SD₅₀, which decreased sharply from pediveliger to D₅₀. Overall, data to develop the environmental risk assessment for these future environmental pollutants will be presented.

Effect of herbicides on embryo-larval development and metamorphosis in the Pacific oyster, Crassostrea gigas

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The summer mortality syndrome which sporadically threatens oyster farming appears to be due to a combination of several intrinsic and extrinsic factors including environmental factors related to reproduction and xenobiotic stress. In this context, terrestrial inputs including pesticides could be involved in oyster mortality events, not as a single causative agent but as additional stressors. The study was carried out in the frame of the European Project Chronoex. The aim of this project was to evaluate the impact of different contaminants (including pesticides) used in the region surrounding the English Channel on the early stages of Crassostrea gigas. Experiments were conducted on oyster spat, a model sensitive to environmental contamination by xenobiotics and used as a keystone species to test different environmental conditions. The effects of these substances and copper sulphate were studied in D-hinged larvae in order to assess their potential embryotoxicity by comparing the sensitivity of normal larval development related to the kind of abnormality (multi-variated analyses). The effects were also researched in pediveliger larvae ready to metamorphosis (calculations of metamorphosis rates). For metamorphosis test, we optimized Cooch & Bonnar’s protocol because of the difficulty to count moving larvae. For each endpoint (embryo larval development and metamorphosis rates), results of 4 experiments allowed us to determine the EC₅₀ values. As regards embryotoxicity, 2 kinds of Roundup appeared about 10-fold more toxic than glyphosate and AMPA and the toxicity of 2M4C reached 3.5-fold of those active substances. In the metamorphosis assay, a similar result was recorded and, glyphosate, AMPA and mecoprop appeared little toxic (EC₅₀ >100 mg L⁻¹). Finally, the comparison between the 2 larval stages generally showed a greater sensitivity of D larvae (embryotoxicity) compared to pediveliger larvae. For most of the molecules, results showed that the toxicity of Zn and insecticides increased with the larval stage. This is not the case for the SD₅₀, which decreased sharply from pediveliger to D₅₀. Overall, data to develop the environmental risk assessment for these future environmental pollutants will be presented.

ECotoxicity of nitramines, important transformation products of amines used in carbon capture

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Due to the imminent use of amines in the CO₂ capturing process from gas power stations in Norway, it is anticipated that the inputs of amines and transformation products into the environment will increase. The two main groups of transformation products that have the potential to cause environmental harm have been identified as nitramines and nitramines, both of which are considered to be carcinogenic. Based on the theoretical model described in laboratory experiments and field observations, we found nitramine compounds, 2-(nitroamine) ethanol (CAS: 74386-82-6) and dimethylnitramine (CAS: 4164-28-7) to be present. However, despite the likelihood of these compounds increasing in the low to mid mg/L range for all aquatic toxicity tests. In addition, the sub-lethal effects of these compounds was also investigated, using in a 3 tiered approach to assess the potential mutagenic and carcinogenic effects of these compounds. Overall, data to develop the environmental risk assessment for these future environmental pollutants will be presented.

Effects of arsenic on physiological parameters of mussels (Mytilus edulis) from the Scheldt Estuary (Belgium)

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Mussels are marine organisms widely used as environmental biomonitorers, due to the ability to concentrate pollutants in their tissues with minimal metabolic transformations.
Arsenic is widespread in the marine environment and occurs in various chemical forms, principally as arsenate (As V) and to a lesser extent as arsenite (As III), which is a more toxic form of arsenic. Arsenic introduced into the environment often contaminates aquatic ecosystems, where the residence time has been estimated at about 50 years. This study aimed to determine whether exposure to specific heavy metal arsenic has an effect on the physiology of mussels. The effect of arsenic in mussels was analyzed in an exposure study in which six concentrations of As(III) and one control for 10 days, during which period several physiological parameters were analyzed (Scope for Growth, energy reserves, body condition index, Mts and arsenic accumulated by the mussels). Arsenic does not seem to affect the physiological parameters of Mytilus edulis, but the organisms can accumulate As (III) proportionally to the concentrations that it is exposed, especially at high concentrations. Several concentrations used in the present study were not lethal to the mussels, but after 10 days of exposure some parameters (lipids and SFG) have positive correlation with arsenic in sea water, suggesting an effect of arsenic. Long-term experiments should be performed to really understand these effects.

TU 376

Marine ecosystem health assessment through the Integrative Biological Index (IBI) in mussels from Portugal NW and Basque coasts

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In order to assess the biological effects of pollution, mussels Mytilus galloprovincialis, were seasonally sampled in seven localities subjected to different levels of pollution along the North Iberian Peninsula Coast (NIPC) over one year (April, June, October 2010 and in February 2011); 4 in the NW Coast of Portugal (Vila Chã, São Bartolomeu do Mar, Viana do Castelo and Carreço) and 3 in the Basque Coast (Arrietxe, Gorliz, Mundaka). In each locality, a battery of biochemical and cellular and tissue-level biomarkers were applied: acetylcholinesterase (AChE) and glutathione S-transferases (GST) enzymatic activity, levels of lipid peroxidation (LOP), lysosomal enlargement (lysosomal volume fraction of the cell membrane) and metabolic activity (LDH), cell type replication (volume density of basophilic cells), VcRAs in digestive gland epithelium, and changes in the morphology of digestive algae (mean luminal radius to mean epithelial thickness MLR/MET). These biomarkers were integrated in the Integrative Biomarker Index (IBI), which was developed within the framework of the Prestige oil spill (POS). The IBI index revealed that mussels’ health was affected in chronically polluted localities. Star plots accompanying IBI provided complementary information concerning mechanisms of biological response to environmental insult. The NIPC is a high risk area for marine animals due to intense marine traffic. This is an important integrative field study to understand the present environment health status and to determine the levels of general stress on core localities which provide reliable assessment of future impacts of pollution. This study was supported by the Government of the Basque Country through a post-doctoral grant to L. Garmendia (ref. IF10.244) and K-EKORITZEN project and by the Portuguese Foundation for the Science and Technology and FEDER funds through the project RAMOS (ERANP0001/2007; EU AMPERA ERA-NET; ERAC-CT2005-01165).

TU 377

Metal exposure and associated effects in mussels: an integrated approach - hypotenated analytical techniques and biomarkers

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Aim of the study was the assessment of metal exposure in coastal areas under indirect or direct influence of maritime activities and nautical tourism (port and marinas) using integrated metal and analytical approaches in bioindicators species (M. galloprovincialis). Next to the sources of pollution, a large number of metals are present in the environment as a result of human activities. The ban of organo-tin compounds, Cu is the main contaminant of heavy metals in coastal areas of the Atlantic coast of Spain. Significant differences were observed between the two lagoons regarding the mercury burdens accumulated and the enzymatic activities of Glutathione S-transferase, glutathione peroxidase and acetylcholinesterase activities in mussels transplanted to harbour areas.

In this study we applied new methodological approach - investigation of metal distribution in the cytosolic fractions of proteins separated by size exclusion chromatography (SEC-HPLC) and detected by inductively coupled plasma mass spectrometry (ICP-MS). Additionally, the cellular energy allocation (CEA) methodology was applied as a general biomarker of physiological status of mussels. Supplementary, a non-target bioanalytical approach utilizing 2D gel-electrophoresis will give hints for further potential protein biomarker. Thus, this study represents a contemporary and new approach in quantification and assessment of the environmental risk caused by metal exposure, as well as the potential toxic effect of metals. The study was performed on indigenous mussel populations sampled in the middle part of the eastern coast of the Adriatic Sea at the locations of two ports, two marinas and a reference (unpolluted) site. The results obtained from the sampling in March 2011 will be presented. This study was performed within the scope of the DAAD project (Project-ID 50732021 Deutscher Akademischer Austausch Dienst) during which scientific collaboration between the research groups from Croatia and Germany was established.

TU 378

An improved on-line clam-based behavioral monitoring model allows an approach to estimate dose-response profiles of clams exposed to waterborne copper

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The aim of this study was to develop an improved on-line clam-based behavioral monitoring model. The system included a valve apparatus and three kinds of valve closure action analytic programs that can offer a real-time and cost-effective method to construct an approach for estimating dose-response profiles of clams (Corbicula fluminea) exposed to waterborne copper (Cu). The valveometric technique can reduce the environmental stress of observed bivalves to promote a measuring precision in the spontaneous status of recording daily valve movements. The clam-based on-line behavioral response monitoring system was used to respectively monitor the valve closure responses of C. fluminea exposed to unpolluted environment and various Cu concentrations. In this study, a probabilistic-based approach describing the valve behavioral response of C. fluminea exposed to unpolluted environment and Cu was developed. The magnitudes of shell gap of 20 and 50% were respectively adopted as the determining thresholds of the closing (VC) and siphon extending (SW) extensional behavior status to digitalize the valve movements in both the time-varying dose-response profiles (RVC and RSW) based on an empirical three-parameter Hill model. It also allowed the estimation of the integration time-specific EC50 and EC90 values as a bioassay approach. The daily valve opening and closing rhythm were characterized by a three-parameter lognormal function. The time-specific EC50 and EC90 values were estimated using online monitoring data. The integration of Kaoru models for the daily valve opening and closing rhythmic functions were estimated at integration times of 10, 15, 30, 60, 120 and 300 minutes. The results revealed that the 90% based valve behavioral observation has a better sensitive response for detecting a lower waterborne Cu concentration than that of RVC within one hour. The results also demonstrated that the response sensitivity of clams exposed to Cu depended on the initial valve status with the daily valve opening and closing rhythmic functions. The present study has shown that dose-response relationships of clams exposed to waterborne copper can be taken into account the related response characteristics of valve activities to promote the predictive capabilities of this bioassay approach.

TU 379

Glutathione S-Transferase, glutathione peroxidase and acetylcholinesterase activities in mussels transplanted to harbour areas

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Detectable levels of heavy metals (Pb, Cd, Cu, Zn) in sediments and mussel tissues from Vigo municipality (Galicia, NW Spain) were responsible for a pilot study field exposure of coastal mussels Mytilus galloprovincialis to acute Cu and Zn concentrations. Acetylcholinesterase (AChE) and glutathione S-transferase (GST) activities in mussel gills and digestive gland were measured. Additionally, glutathione peroxidase (GPx) activities were measured in the gills of transplanted mussels (n=12). GST activity is specifically inhibited by organic pesticides such as organophosphates and carbamates. The concentrations of major contaminants (trace metals, polychlorinated biphenyls and polyaromatic hydrocarbons) accumulated in the mussels tissues were also measured. Mussels from the most polluted sites consistently exhibited a significant increase in GST activity compared to the controls. Both biomarkers showed the same pattern of exposure and toxicity. The present study has shown that GST and AChE activities can be used as potential biomarkers of toxicity for active monitoring in marine coastal ecosystems.
Kairomones are signs produced by the predator and that can be detected by the prey, causing stress and consequent fitness costs. In order to measure those costs and evaluate the effects of simultaneous exposure to multiple stressors, the sea snail Gibulla umbilicalis was exposed to increasing concentrations of mercury and salinity and/or predatory chemical effluents

TU 381
Impact of suspended particles on bioavailability of petrogenic PAH in cod (Gadus morhua), mussels (Mytilus edulis) and passive samplers exposed to produced water M. Pampmann1, C. Björklöf1, C. Harmst1, S. Vingen1, B.F. Godal1, R.C. Sundt1

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Caged organisms and passive samplers are routinely used to monitor environmental impact of operational discharges from offshore oil and gas production. Comparison of experiments can be challenging if different risk assessment models may be required. The present study was designed to study the influence of particulate matter (PM) on the bioavailability of hydrocarbons in produced water (PW). Our results have shown that the combination of biotic or predator-specific compounds induces

TU 382
Estimation of experimental conditions to maximize mussel shell capability in trace metal accumulations A. Zuin, S. Manente, G. Ravagnan

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The estimation of mussel shell capability of concentrate heavy metals was performed by means of a set of laboratory experiments conducted with mussels collected in unpolluted sites exposed to sub-lethal concentrations of a known toxic metal. Multiple cycles of accumulation and depuration were carried out all along the experimental period; the constant monitoring of experimental conditions and periodical metal determination in shell and tissue samples allowed the individuation of the accumulation and depuration patterns in both Bivalves materials in the selected species. Alternative exposure to contaminated and clean seawater respectively was performed in order to record the different metal concentration trends in shell and soft tissues. In fact it is demonstrated that once the metal is incorporate in the crystalline carbonate lattice, his loss from the shell during mussels lifestage can be considered of no concern; on the other hand it is known that soft tissues are able to release contaminants in response to changes in environmental conditions. The aim was to establish a set of experimental conditions able to optimize metal accumulations on mussel hard parts and to calculate the specific MATC (Maximum Acceptable Toxic Concentration) value. So it was possible to set physical, chemical and physiological parameters in order to maximize the shell metal content and set the implementation for bioinoculation strategies exploiting mussels metal activity and detoxification mechanisms.

TU 383
The effect of sublethal nickel concentrations on Mytilus galloprovincialis: a multiple biomarker study L.J. Dallas1, T.P. Bean1, A. Turner1, B.F. Lyons1, A.N. H2

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Nickel is a known mammalian carcinogen and mutagen, typically found at low concentrations in the marine environment (<1 µg l-1), but enriched at coastal locations near mining operations. The aim of this work was to use a multiple biomarker approach to assess the effects of nickel on mussels. Nickel-acclimated and SPMD only mussels were exposed for 17 days to diluted PW in a continuous flow-through system. The dilution was set at 0.1%, from day 6 until day 10 the flow was set to 0.2% to mimic real field conditions with varying PW concentration over time. The exposure set up included: a negative control with sea water only, a positive control with diluted PW only and three tanks containing diluted PW and algae mix at low, medium and high concentrations. The following parameters were analysed: PAHs in PW; PAH concentrations in SPMD and mussel soft tissues, nickel and PAH metabolites in fish. Due to the important role of gills for absorption of waterborne PAHs, a histophatological investigation of fish gills was conducted as a support parameter. The presence of realistic densities of organic particulate has only minor impact on the bioavailability of low molecular weight PAHs in fish, mussels and passive samplers. Bioavailability of 3-ring PAHs was only significantly reduced in mussels when algae particle density was high (about 50000 cells/ml).

TU 384
Biomarkers responses of the native clam Scrobicularia plana and changes of macrobenthic functional composition in a shallow tidal creek affected by fish aquaculture effluents G.V. Aguirre-Martinez1, C. Silva1, M. Mattioli2, E. Fabbrini2, A.T. del Valls2, M.L. Martín-Díaz1

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The effects of solid organic wastes from a marine fish farm on sediments were tested using biomarkers in native clam (Scrobicularia plana) as biochemical indicators and macrobenthos as ecological indicators. The clams and macrobenthic samples were collected in the intertidal sediment in October 2010 from five sites of the Rio San Pedro creek, following a gradient of contamination from the aquaculture effluent to the control site. Phase I and Phase II detoxification enzymatic activities (ethoxyresorufin O-deethylase (EROD) and glutathione-S-transferase (GST)), oxidative stress parameters (Lipid Peroxidation (LPO) and DNA strand breaks) were measured in clams’ digestive gland tissues. Numbers of species, abundance, richness and Shannon diversity indexes were the biodiversity indicators measured in macrofauna. In parallel, redox potential, pH and organic matter in sediment, and dissolved oxygen in the water column was measured in situ. Significant (p < 0.05) increases of GSH, enzymatic activity and antioxidative response were observed in the areas close to aquaculture effluents. Biomarkers (DNA, LPO and GPX) were significantly (p < 0.01) negatively correlated with pH, redox potential and dissolved oxygen and positively correlated with organic matter. On the contrary, macrobenthic biodiversity were significantly (p < 0.01) positively correlated with redox potential and dissolved oxygen and negatively correlated with organic matter. It has been demonstrated that effluents from fish aquaculture activities in Rio San Pedro creek may induce oxidative stress in soft-sediment species which may lead to alteration of the biodiversity and health status of the exposed organisms.

TU 385
Mechanisms of acute toxicity of metals in estuarine and marine invertebrates A. Pament, T.L. Estensen

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In freshwater invertebrates, acute metal toxicity is generally associated with the disruption of ionic and osmotic regulations. In contrast, the mechanisms involved in acute metal toxicity in mussel and marine invertebrates are not well characterized and the research on the possible mechanisms involved in the acute toxicity of metals in these animals was reviewed. Data on the acute effects of metals (copper, silver, zinc, and nickel) on biochemical and physiologic biomarkers in estuarine and marine invertebrates (foraminifers, sea urchins, sea anemones, sea cucumbers, copepods, isopods, shrimps, crabs, and tunicates) were compiled, and in the present study the most recent information gathered by our research group on the mechanism involved in the acute toxicity of metals in these animals were reviewed. Data on the acute effects of metals (copper, silver, zinc, and nickel) on biochemical and physiologic biomarkers in estuarine and marine invertebrates (foraminifers, sea urchins, sea anemones, sea cucumbers, copepods, isopods, shrimps, crabs, and tunicates) were compiled, and in the present study the most recent information gathered by our research group on the mechanism involved in the acute toxicity of metals in these animals were reviewed.

TU 386
Withxic and abiotic factors increase the toxicity of contaminant-enriched environments in exposed sea snails? A.S. Cabecinhas1, L. Fonseca1, A.M.V.M. Soares1, J.L.T. Pestana1, F.L. Lemos2

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Biomarkers responses to sublethal metal exposure have recorded an extensive variation due to its impact on human health and wildlife. Currently, concerns about climate changes and the way they might affect the environment are being more and more studied. Moreover predator-prey relations have been progressively more pointed as stress sources. Environmental contamination and biotic and abiotic stress co-occur, thus it is important to assess and understand the synergistic or antagonistic effects of these changes (e.g. salinity) and/or predation combined with common environmental contaminants. Kaemones are signs produced by the predator and that can be detected by the prey, causing stress and consequent fitness costs. In order to measure those costs and evaluate the effects of simultaneous exposure to multiple stressors, the sea snail Gibulla umbilicalis was exposed to increasing concentrations of mercury and salinity and/or predatory risk, simulated by a concentration gradient of kairomones produced by a sea snail predator - sea star. Mortality, feeding rates (indirect measure by post-feeding exposure egestion) and avoidance behaviour were assessed. In the end of the experiments, animals were sacrificed and cholinesterases' activity and energy reserves were measured. Our results have shown that although responses could be triggered by single stressors, the combination of biotic or predation-specific compounds induces
Effects of mercury contamination on acute and chronic parameters of exposed sea snail Gibbula umbilicalis

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Mercury is a common contaminant in the environment, having natural sources, such as volcanism, and anthropogenic origins, such as mining tailings or industrial effluents. Exposure to some environmental contaminants, even for short periods of time, may cause serious damage to exposed individuals. Thus, to evaluate the possible effects of mercury in the sea snail Gibbula umbilicalis, acute and chronic assays were performed by exposing the animals to increasing doses of the contaminant for 96 and 168 hours.

Mortality, feeding rates (indirect measure by post-feeding exposure egestion) and avoidance behaviour were assessed. In the end of the experiments, animals were sacrificed and cholinesterases’ activity and energy reserves were measured.

Although evaluated at individual level, the effects observed here might be translated to the population and community level, since feeding impairment and change in energy allocation may have effects on the organisms’ fitness, and reproductive performance. Moreover, modification in cholinesterases’ activity and its common link to behaviour shifts might lead to important implications in population dynamics.

Results have shown that most of the parameters evaluated tend to be impaired by this compound, showing the deleterious effects that this compound may have at the population level and on marine life.
contaminants. Since they represent a very large biomass, they can act as an important sink for contaminants becoming a gateway for higher trophic levels. Moreover, it is known that macroalgae, especially estuarine, are very important in the aquatic system due to several aspects: easy to find and to identify, vast biomass percentage and great biocompounds, making them potential sentinel of to monitor xenobiotics in aquatic environments. Macrocystis presence or absence, as well as their look-like may reflect the current state of the marine ecosystem, thus it is mandatory to develop new tools and methodologies to quickly and cost-effectively assess their status.

The green macroalgae Ulva lactuca was used as a model species. When exposed to xenobiotics, can show signs of decay by losing their colour and their texture. To assess these changes, several parameters such as growth rate (cell count) and photosystem II efficiency (Pulse Amplitude Modulation fluorometry) after 4.5 hours as a measure for toxicity. A difference in species sensitivity was observed with anthropogenic compounds and the natural toxin Decadienal were tested according to the Toxic Unit (TU) concept. Reduction in effective photosystem II (PSII) efficiency was coupled to gas chromatography tandem mass spectrometry (GC-MS/MS) for determining the effect of a Cu+Zn mixture on the marine alga Dunaliella tertiolecta, Phaeodactylum tricornutum and Thalassiosira pseudonana were exposed to two anthropogenic compounds (Irgarol and Tributyltin) and two natural toxins (Decadinal and microcystin). For D. tertiolecta binary equitoxic mixtures of the anthropogenic compounds and the natural toxin Decadinal (TU) concept were tested according to the Toxic Unit (TU) concept. Results showed that photosystem II (PSII) efficiency was determined by Pulse Amplitude Modulation (PAM) fluorometry after 4.5 hours as a measure for toxicity. A difference in species sensitivity was observed with T. pseudonana being the most sensitive species. Irgarol is the most toxic mixture for both species and the natural toxin Microcystin is the least toxic. Both natural toxins are clearly less toxic to the tested algae. For D. tertiolecta the effect of two mixtures: a small additive mixture effect was observed for Decadinal and TBT, while a large more than additive mixture effect was found for Decadinal and Irgarol. These results suggest that single compound toxicity data can not be used to predict the mixture toxicity of these compounds. A species, compound and mixture specific response was observed for all tested algal species and the combination of toxicity determining the toxic pressure of coastal waters on the primary producers.

The use of protozoa in ecotoxicology: from biological model to environmental biomonitoring application
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Nowadays, anthropogenic activities have resulted in accumulations of inorganic and organic pollutants in the environment as well as in the food chain, hence leading to serious ecological and human health problems. This may pose a risk to benthic and epibenthic organisms and it is crucial to develop tools that will identify adverse effects of contaminants on benthic environments as well as to toxicity mechanisms to biological effects. Due to their nature as a ciliates cellorganism and their position in the food web, ciliated protozoa are suitable models for evaluating the biological effects of chemicals in living organisms as well as in estimating pollution levels in aquatic environments. Lethal and sub-lethal effects of exposure to inorganic and organic pollutants were tested on the cell mortality, replication rate, lysis percentage and endocytosis rate of the marine ciliate E. crassus. Increasing concentrations of mercury, copper, and benzo(a)pyrene , individually and as mixtures, were investigated in our study as they might be bioavailable in naturally occurring polluted sites. Exposures to binary mixtures of all studied pollutants were performed showing both inorganic-organic and inorganic-inorganic additive and/or antagonist effects. Moreover, medium salinity was also varied to mimic estuarine-like environmental conditions in response to toxicological laboratory experiments performed under de short time and simplicity of the test procedures, the use of protozoa such as E. crassus is promising and can be used as bioindicators for evaluating the toxicity of different environmental matrixes like pore water, sediments and elutriates. Can foraminifera be reliably used as environmental indicators?
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Foraminifera are promising marine indicators due to the specific occurrence of a certain class of pollutants in both sediments and elutriates. Overall outcomes were able to discriminate a gradient of stress in the four investigated sites. Furthermore, biological responses were linked to the specific occurrence of a certain class of pollutants in both sediments and elutriates.

Toxicity of a Copper and Zinc mixture on Dunaliella tertiolecta
University of Amsterdam, Amsterdam, Nederland

Toxicity experiments with one compound, but contamination in the field generally consists of mixtures of contaminants. As a result of this study it was therefore to determine the effect of a Cu+Zn mixture on the marine alga Dunaliella tertiolecta. Growth rate (cell count) and photosystem II efficiency (Pulse Amplitude Modulation fluorometry) of D. tertiolecta were determined after three days of exposure to Cu, Zn and equitoxic mixtures of Cu+Zn, composed according to the Toxic Unit concept. Our results showed that growth of D. tertiolecta was more sensitive to Zn than to Cu. Photosystem II efficiency was less sensitive to the metal(mixtures) than growth.
The results highlight that protein ubiquitination assessment represents a promising approach to evaluate and gain insight into stress response in marine ecotoxicology. For a highly sensitive endpoint: a 72h-inhibition of autotrophic growth rate was calculated according to standards methods. No observable effect concentration (NOEC) values were 2.53, 3.48 and 12.5 µg L⁻¹ for BDE-47, 99 and 154, respectively. The calculated IC₅₀ (the concentration inhibiting growth rate by 50%) corresponded to 9.3, 12.78 and 54.6 µg L⁻¹ for BDE-47, 99 and 154, respectively. The 50% inhibitions of growth rate (IC₅₀) values were: 25.7 µg L⁻¹ BDE-47, 30.5 µg L⁻¹ BDE-99 and 243.7 µg L⁻¹ BDE-154. Significant (p < 0.05) adverse effects were observed for all compounds at concentrations > 15 µg L⁻¹. Our results indicate that under laboratory conditions PBDEs were acutely toxic to seawater algae at concentrations near 10 µg L⁻¹. However, further work is required to investigate long-term effects in these and other aquatic organisms.

Concern around contamination of the aquatic environment by polycyclic aromatic hydrocarbons (PAHs) is high due to their toxic, carcinogenic, mutagenic and/or teratogenic effects. Fluoranthene (FLU) is a priority PAH that is commonly detected in sediments, water and biota of European estuaries. It is persistent and an important lipophilic contaminant. Proteins whose expression and/or ubiquitination level are affected by crab location will be subjected to identification by mass spectrometry, in order to tentatively elucidate the mechanisms involved in pollutant response. Does contamination impact the shore crab (Carcinus maenas) resistance to an additional stress? An insight through a multiparametric approach. We investigated the accumulation of FLU and its metabolites in the shore crab Carcinus maenas in relation to oxidative stress. These results highlight that protein ubiquitination assessment represents a promising approach to evaluate and gain insight into stress response in marine ecotoxicology.
In situ assays based on feeding depression have been proposed as sublethal assays which allow to assess direct and immediate contaminant effects on key ecosystem functions (e.g. organic matter decomposition, grazing), long before direct effects on individuals (e.g. growth) are extrapolated to effects at higher levels of biological organization - the so-called trophic cascades. This study was undertaken to assess the feeding depressions and resulting sublethal effects of PAHs on the estuarine isopod Cyathura carinata. In a short-term in situ assay based on the postexposure feeding of the estuarine isopod Cyathura carinata, the objective of the present study was to develop and evaluate a short-term cost-effective method in situ sediment assay based on the postexposure feeding of an estuarine species widely distributed and with an important ecological role in estuarine foodwebs. The selected species was the benthic isopod Cyathura carinata, a secondary prey, feeding on fish and birds, occurring along the North Atlantic coast and in the Baltic and Mediterranean seas. The methodology for feeding quantification was first developed and optimized under laboratory conditions, selecting sublethal test endpoints that are less affected by laboratory conditions, which is particularly relevant for estuarine ecosystems where environmental conditions are highly variable. In this context, the objective of the present study was to develop and evaluate a short-term cost-effective method in situ sediment assay based on the postexposure feeding of an estuarine species widely distributed and with an important ecological role in estuarine foodwebs. The selected species was the benthic isopod Cyathura carinata, a secondary prey, feeding on fish and birds, occurring along the North Atlantic coast and in the Baltic and Mediterranean seas. The methodology for feeding quantification was first developed and optimized under laboratory conditions. In situ assays provide a more realistic scenario than assays conducted under laboratory controlled conditions, which is particularly relevant for estuarine ecosystems where environmental conditions are highly variable. In this context, the objective of the present study was to develop and evaluate a short-term cost-effective method in situ sediment assay based on the postexposure feeding of an estuarine species widely distributed and with an important ecological role in estuarine foodwebs. The selected species was the benthic isopod Cyathura carinata, a secondary prey, feeding on fish and birds, occurring along the North Atlantic coast and in the Baltic and Mediterranean seas. The methodology for feeding quantification was first developed and optimized under laboratory conditions.

The biotransformation products from PAH exposed N. diversicolor and N. Virens were collected by extracting the test water of laboratory mesocosms exposures at different times. The water soluble biotransformation products were extracted and concentrated by SPE, and their toxicity (EC50) were determined by exposing Daphnia magna to dilutions of these extracts. The water soluble biotransformation products were identified and quantified by UHPLC connected in series to a fluorescence detector and a Quadruple Time of Flight Mass Spectrometer. To aid the identification enzymatic deconjugation of the phase II products was performed, and identified by use of GC Mass Spectrometry (GC-MS).

This work presents a novel approach to test the toxicity of transformation products of a contaminant mixture, where organisms are exposed to the entire range of biotransformation products. Also, this work shows the changes in toxicities of the transformation products as transformation of the PAHs progresses, and gives insight to the mechanisms of biotransformation of alkylated PAHs.

In this experiment show that Vitellogenin gene expressions were more sensitive than the ZR, under normal conditions in the environment. Future studies will be focused on understanding the reproductive cycle and the genetic variability of these fish.

The Portman area (Cartagena, SE Spain) is one of the areas most heavily polluted by metals in the western Mediterranean Sea and highly impacted by emissions from oil-related and chemical industrial activities in the adjacent Escombreras Valley. An integrated assessment was conducted of the health status of red mullet (Mullus barbatus) from this area. Concentrations of 2PBDs ranged from 37.3 to 218 ng g\(^{-1}\) lipid weight (lw) for sea bass in Bitezage Lagoon, whereas in the Mediterranean Sea, concentrations ranged from 20.8 to 36.6 ng g\(^{-1}\) lw. BDE-47 was the major congener in samples from the two areas. Mean levels of MeO-PBDs ranged from 49.4 to 788 ng g\(^{-1}\) lw in sea bass, and the mean concentration of these naturally produced organochlorines was 482 ng g\(^{-1}\) lw. The total PBDs and total MeO-PBDs concentration in sea bass from Bitezage Lagoon was similar or slightly lower than those reported for other species from other locations around the world. No significant correlations were found between the organohalogen compounds levels and the fish characteristics.

In this study, the effect of PAHs on red mullet (Mullus barbatus) from the priority polluted Mediterranean area of Portman (Cartagena, SE Spain) was assessed. The major headspace analyses of the Mediterranean Sea and highly impacted by emissions from oil-related and chemical industrial activities in the coastal Escombreras Valley. An integrated assessment was conducted of the health status of red mullet (Mullus barbatus) from this area. Concentrations of 2PBDs ranged from 37.3 to 218 ng g\(^{-1}\) lipid weight (lw) for sea bass in Bitezage Lagoon, whereas in the Mediterranean Sea, concentrations ranged from 20.8 to 36.6 ng g\(^{-1}\) lw. BDE-47 was the major congener in samples from the two areas. Mean levels of MeO-PBDs ranged from 49.4 to 788 ng g\(^{-1}\) lw in sea bass, and the mean concentration of these naturally produced organochlorines was 482 ng g\(^{-1}\) lw. The total PBDs and total MeO-PBDs concentration in sea bass from Bitezage Lagoon was similar or slightly lower than those reported for other species from other locations around the world. No significant correlations were found between the organohalogen compounds levels and the fish characteristics.

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Characterization of selected Metals in United Arab Emirates coastal fish and locally produced vegetables.

Sediments experienced by macrofaunal polychaetes. Subsequent adult mortality and the numbers and timing of spawning events were quantified as well as the effects on mechanisms of reproductive impairment of hypoxia remain unclear. Using primary cell culture of marine medaka (Oryzias melastigma) testis as an in vitro model, experiments study of enzymatic, histopathologic and genotoxic biomarkers using biomarkers from fish liver for assessment in Tunisia coastal waters.

Cycle of N. virens through spiking sediment to a range of nominal concentrations (50, 500 and 1000 mg kg⁻¹ dry weight of sediment) chosen to reflect those present in investigations as gametogenesis and spawning of of a population are highly synchronized. Heavy metals interact with sediment in many ways and spiked sediment has been colonization of polluted sediments.

Our findings suggest that the pristine size of the dispersed particles affect the bioavailability and the overall toxicity.

Toxic effects of ZnO nanoparticles towards marine algae Dunaliella tertiolecta

Dose response curve and population growth rate alterations deriving from the exposure to ZnO nanoparticles of the marine alga D. tertiolecta were evaluated. Bulk ZnO and toxicic ions were also investigated for comparison of aggregation state and particle size distribution monitored during the experimental testing time. The results show that nZnO is more toxic (EC50: 2.42 (0.97-5.36) mg L⁻¹, NOEC: 0.01 mg L⁻¹) than its bulk counterpart (EC50: 4.45 (3.45-5.98) mg L⁻¹, NOEC: 1 mg L⁻¹). Cross-referencing the toxicity parameters calculated for ionic zinc (EC50: 0.65 (0.36-0.70) mg L⁻¹, NOEC: 0.01 mg L⁻¹) and the dissolution properties of the ZnO it can be gather that nZnO is more toxic (EC50: 2.42 (0.97-5.36) mg L⁻¹, NOEC: 0.01 mg L⁻¹) than its bulk counterpart (EC50: 4.45 (3.45-5.98) mg L⁻¹, NOEC: 1 mg L⁻¹). The higher toxicity of nZnO cannot be ascribed exclusively to free zinc ions. Nonetheless growth rates of D. tertiolecta were not significantly affected by nZnO exposure. Our findings suggest that the pristine size of the dispersed particles affect the bioavailability and the overall toxicity.

Effects of hypoxia on spermatogenesis and steroidogenesis of marine medaka (Oryzias melastigma) testis: an in vitro study

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Inhibition of GST, GPs and AChE activities in marine mussels by exposure to the dissolved polybrominated dibenyl ethers BDE-47
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Response times vary between metals with amphipods exposed to copper producing behavioural changes and mortality at a faster rate than exposure to cadmium, lead or

As traditional bioassay methods use probit analysis for individual period endpoints treated independently, a new statistical model that combines bioassay model with a

Spatial distribution and accumulation patterns of cyclic methyl siloxanes (cVMS) in fish from Northern Norway
A. Wärnér1, T. Nøtst2, G. Christensen3
1 Norwegian Institute for Air Research, Tromsø, Norway
2 Akvaplan-niva, Tromsø, Norway
3 University of Bergen, Bergen, Norway

Organochlorine pollutants in oceanic migratory birds: influence of body condition in PCBs and DDTs concentrations among different tissues
F.L. Colabuono1, S. Taniguchi, R.C. Montone
1 Oceanographic Institute - University of São Paulo, São Paulo, Brazil

Alturtoxes and petrels (Procellariiformes) are migratory oceanic birds of considerable conservational interest. Polychlorinated biphenyls (PCBs) and organochlorine pesticides

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in this location oxidative stress biomarkers are the main contributors in the IBR outcome. Sediments in the commercial harbor (site 4), presented higher Cd and Cu concentrations associated to organic contaminants. The IBR pointed this site as the second most stressed location and antioxidant enzymes had a great contribution. On the other hand, site 2 (outer basin of the harbor area) was considered the most stressed location, a loss of integrity of the ecosystem, and as a consequence, a loss of biodiversity and a potential risk for human health. In the specific case of organisms that have a great international concentration-based and risk-based criteria revealed that PAHs in Mugil lisa fishes from Guanabara Bay was not a significant thread to public health. The data from this study was compared to other estuarine and coastal regions in the world reported in the literature. The PAH metabolite concentrations in Guanabara Bay were in the same range of various regions that receives chronic anthropogenic pollution as expected. However, PAHs results in muscular tissue, when compared with three international concentration-based and risk-based criteria revealed that PAHs in Mugil lisa fishes from Guanabara Bay was not a significant thread to public health. To sum up, the data of this study can be used as background for Guanabara Bay ecosystem to PAHs.

TU 422
Polycyclic aromatic hydrocarbons (PAHs) are ubiquitously distributed in the aquatic environments, coming from natural and anthropogenic sources. PAHs can be produced due to anthropogenic activities, such as fossil fuel combustion, boating activities and forest fires. Natural low molecular weight (LMW) PAHs are also known to be produced inside termite nests and by woody plants. Few studies were developed in the Amazon region concerning PAHs sources and distribution. The study area, Guajará Bay, northern Brazil (48°30’ W and 1°30’S), is part of the largest watershed in the world and has great ecological importance due to its vast biodiversity. One of Amazon’s biggest cities, Belem, capitol of the state of Pará, is located in its margins. Most of the sewage produced in the city is released directly into Guajará Bay without any treatment. For this preliminary work, estuarine surface water samples were collected around Belem, near the main input channels. Sediments were soaked extracted and PAH concentrations were analyzed by gas chromatography coupled to mass spectrometry detection (GC/MS). Total PAH concentration ranged from 8.41 to 90.03 g l⁻¹, indicating a low contaminated environment. PAHs are probably diluted and degraded during transport as a result of the area’s strong hydrodynamics. Parental high molecular weight PAHs show higher levels than LMW and alkylated PAHs in all samples. LMW PAHs, which could be correlated to biogenic or petrogenic origins (naphthalene and phenanthrene), were not found in significant concentrations. In most samples, pyrene had the highest concentration among individual compounds, suggesting diagenetic sources. These results, combined with specific compound ratios, suggest that diagenetic and pyrolytic inputs (from fossil fuel burning and specialized forest fires), are the main sources of PAHs to Guajará Bay.

TU 423
Ecotoxicological and chemical parameters as tools to develop an integrated approach in a coastal ecosystem

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As most coastal zones, Venice Lagoon is subject to the deposit of sedimentological materials, in form of inorganic and organic detritus, nutrients and heavy metals, and there is also a strong anthropic pressure; consequently, heavy metal amount is much bigger in lagoon sediments than into overlying water. In fact, remobilisations processes are able to mobilize contaminants into the water column or resuspend inside bottom sediments, modifying also the bioavailability. Processes that could easily change their geochemical parameters will be studied (e.g. redox potential) and make them more bioavailable. On the long term, this complex net of events can cause to the populations living along the coast.

This study starts from the concept of “biomarker” in a multidisciplinary approach aimed to evaluate the “state of health” of some aquaculture sites and of the Mollusca Bivalves growing up into. Model organism for the research is Tapes philippinarum a species of Mollusca Bivalve of the Veneridae family that in recent years has shown an increased economic value and is in need of support in an optic of sustainable development.

In the last years, ecotoxicological markers have been used (e.g. heart activity, lipoxidation, aminolevulinic acid dehydratase, acetylcholinesterase and alkali-labile phosphates) to evaluate the environmental quality of productive sites and also analysis of geochemical speciation have been carried out to assess the actual bioavailability and mobility of abiotic matrix’s pollutants present inside the trophic chain.

TU 424
Effects of Pollutants in aquatic ecosystems of the cross-border of the southwestern of the Iberian Peninsula

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The southwestern of the Iberian Peninsula is characterized by the presence of diverse aquatic environments, estuaries and marshes which environmental situation depends critically on the impact of pollutant substances as consequence of mining activities, increase of population densities, agricultural and industrial activities. A great number of contaminants are treated and eliminated in the waste water treatment plants, but recently the presence of new emergent pollutants has been detected which chemical nature limits the action of the treatment systems.

In order to assess environmental risk and deterioration in these ecosystems analytical tools were developed under the framework of Interreg IIIA project Rise sub projects and Endroxiciotics and Farmacotox to characterize and diagnostic the presence of emergent pollutants. Samplings were carried in marshes and aquatic ecosystems of the cross border area between Portugal, namely Bay of Cádiz, River Guadiana, Ria Formosa Lagoon and Arade River. Core sediments and benthic organisms (clam Scrobicularia plana and phylactea Nereis diversicolor) were sampled. Accordingly, molecular biomarkers based on the characterization of antioxidant enzymes and oxidative damages in biomolecules were analyzed, as well as neurotoxicity and endocrine disruption. Biological answers were integrated with the determination of contaminants in organisms tissues and samples. This methodology applied to give a comprehensive information about protein changes in organisms’ tissues induced by distinct contaminants to identify new biomarkers to be used in environmental quality assessment. The integration of biomarker responses and sediment characterization can be a useful tool for marine pollution monitoring in southwest of the Iberian Peninsula.

TU 425
Water quality assessment in Portimao Harbor

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According to the Water Framework Directive ports carry out specific activities of recognized high economic and social value that in some cases imply heavy hydraulic activities on the water quality. The explicit recognition of the pollutant activities involves the definition of a specific approach to fulfill a good ecological potential. In this context, the European project “PORTONOVO” was developed to standardize methodologies to evaluate and monitor environmental quality in all Atlantic Coast. In Portimao Harbor (province of Faro, Portugal) a biomonitoring survey was carried out in seven sites to assess the quality of water, sediment and biota (mussels Mytilus galloprovincialis). This involved analyses of: a) water: abiotic parameters (temperature, salinity, pH, and dissolved oxygen), nutrients (nitrates, nitrates and ammonium, silicates and phosphates), chlorophyll a and phaeopigments, and turbidity; b) sediments: organic content, metals (Cd, Cu, Ni, Zn, Cr and Pb), and a battery of biomarkers (lipid peroxidation, antioxidant enzymes (CAT, SOD and GPOX), metallothionen, 8-aminoleucinic acid dehydрадат, acetylichemolase and alka-labile phosphates). All data was integrated using a Principal Component Analyses and the IBR (Integrated Biomarker Response). Sediments in the commercial harbor (site 4), presented higher Cd and Cu concentrations associated to organic contaminants. The IBR pointed this site as the second most stressed location and antioxidant enzymes had a great contribution. On the other hand, the site 2 (out basin of the harbor area) was considered the most stressed location, where sediments had higher Cr, Ni and Pb and organisms associated to higher concentrations of several biomarkers. As for upstream of the harbor area (site 6), there was significant impact of nutrients, phaeopigments, turbidity and some abiotic parameters which reflect the impact of a sewage effluent discharge point. Concordantly in this location oxidative stress biomarkers are the main contributors in the IBR outcome. The biomonitoring survey provided an integrated approach to assess the environmental quality of the port area, proving the need to monitor all these descriptors to assess the good ecological status in Port areas.

TU 426
Persistent organic pollutants in Stenella clymene from Northeastern Brazil: a preliminary survey

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The biomonitoring survey provided an integrated response to assess the environmental quality of the port area, proving the need to monitor all these descriptors to assess the good ecological status in Port areas.
Brazil are still scarce, and none is known to be occurring in the Amazon region. The S. clymene dolphin is a deep water species that has only been observed in waters with depths of 250m-5000m or deeper. Thus, it is almost impossible to study the solitary cetacean. In contrast with S. clymene, the dataset is much more complete for the S. clymene. Still, the population is limited to the western Pacific region, and the data are spotty and fragmentary. The data presented here is based on a single report, but the general situation is unknown. Based on the limited and fragmentary data, it is suggested that the S. clymene is currently not threatened with extinction. However, further studies are needed to fully understand the conservation status of this species.

The study of the genetic diversity of the S. clymene dolphin is also important for understanding the evolution of this species. Although the data is limited, it suggests that the S. clymene has a relatively high level of genetic diversity. This is important for understanding the evolutionary history of the species and the processes that have shaped its genetic makeup.

In conclusion, while the S. clymene dolphin remains a mysterious and elusive species, the data presented here provides important insights into its biology and conservation status. Further research is needed to fully understand this fascinating species and to ensure its long-term survival.
The Lake Shihwa sediments were examined for contamination using three bioassays: survival of amphipod (Monocorophium aetherum) for bulk sediments, fertilization of sea urchin (Stronglyocentrotus purpuratus) for porewater, and bioluminescence of marine bacteria (vibrio fischeri) for sediment extracts. The Lake Shihwa is an artificial Lake which was created by a 12.7 km long dyke for water supply to industrial complex and agriculture area near the city. In the Lake Shihwa region, the cities of Shigueung and Ansan (total population exceeds 1 million) and large industrial complexes (total industrial area is ~31 km²) are located. The survival of amphipod for 30 sediment samples ranged from 75 to 100% (median: 95%) and the fertilization of sea urchin ranged from 1 to 100% (median: 42%) and the bacterial bioluminescence ranged from 12 to 631 mg/L (effective concentration 50%; EC50). The lake sediment contamination was low level. In particular, the survival of amphipod was higher than 80% at all stations, indicating no acute toxicity. High toxicity of sea urchin fertilization, less than 10% of the control sample, was found at all stations in inshore and three stations in offshore. The result suggests that the sediment toxicity was more contaminated in inshore of Lake Shihwa impacted by industrial complexes, compared with offshore containing an outfall of wastewater treatment plants, River, and marine surface sediments. The correlation among the bioassays presents a negatively significant correlation within the invertebrate group. For limpets, such as Polythrichites weddelli and Arctocephalus gazella and liver from Mirounga leonina) the index presented significant correlation within the invertebrate group. For limpets, PCBs profiles are probably affected by the lighter profile of the seasonal melting, to the point of presenting negative correlation with δ15N , and therefore a lesser trend to be excreted, are the ones presenting positive correlation in liver from the same species grosso modo . For liver, there is a significant correlation with δ15N, corroborating the index. Pinnipeds presented large fluctuation due to nutritional stress made evident by the δ15N . For birds, compounds showing negative correlation in eggs, and therefore a lesser trend to be excreted, are the ones presenting a positive correlation in liver from the same species and genus. For liver, there is a significant correlation with δ15N, corroborating the index. Pinnipeds presented large fluctuation due to nutritional stress made evident by the δ15N . For birds, compounds showing negative correlation in eggs, and therefore a lesser trend to be excreted, are the ones presenting a positive correlation in liver from the same species and genus.
agriculture. During many years TSB has been environmentally affected by domestic and industrial effluents, fishing and boat traffic, and agricultural runoff.

The concentration of metals found in sediments is the result of natural phenomena, anthropogenic activities or the combination of both. Therefore, different normalizing analysis and bioassay indicate that the ecotoxicity of PAHs presents high potential only in the sediment from the Huangpu River mouth, while it presents low potential in most other parts of the seas. The species investigated are three Mysticeti - blue whale (Balaenoptera musculus), fin whale (Balaenoptera physalus) and Bryde’s whale (Balaenoptera edeni) - and four Odontoceti - sperm whale (Physeter macrocephalus), bottlenose dolphin (Tursiops truncatus) and long beaked common dolphin (Delphinus capensis). All the free-ranging specimens were sampled using the non-destructive technique of the skin biopsy where the subcutaneous blubber was used for the PAH analyses.

The results showed that there is a statistically significant difference in the accumulation of these compounds in the blubber of these animals. Overall, the Odontoceti showed lower PAH levels than the Mysticeti and, in both suborders, the accumulation seems to be correlated with the size of the species. Furthermore, comparing the PAH fingerprints of the different species, it is possible to note that Odontoceti has a predominance of low molecular congeners (~85%) compared to high molecular ones (~15%), while in Mysticeti the comparison is almost the same: ~35% of low molecular and ~45% of high molecular PAHs.

A biological index of the interactions between metals enrichment factors and sediments from Todos Santos Bay, northwest coast of Baja California, Mexico A. Maltese, D. Coppola, S. Maltese2, C. Panti, L. Rochas-Bragh1, J. Urbani, M.C. Fossi, L. Marsili2

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Developing ecotoxicological methods for relevant test species in coral reef environments

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There is a paucity of applicable ecotoxicological data for marine tropical systems and applicability of toxicity data from species has been widely questioned and criticised. The urgent need to rectify critical gaps in our understanding of ecologically acceptable concentrations of contaminants for the protection of tropical marine ecosystems is widely acknowledged. Research studies have shown that coral species are extremely sensitive to toxicants, however there are many challenges to developing standard toxicity tests using corals.

Our group had been working with the sea anemone Aiptasia pulchella (Carlgren 1943, Fautin 2006, currently under review) to develop toxicity tests for commercial use. The development of a standard ecotoxicological test for the introduction of a new standard phytophyll (Cnidaria) to the suite of commercial toxicity tests available for marine waters in Australia. We have developed animal husbandry methods, and ecotoxicological methods for testing contaminants. Results to date show that the sensitivity of this test organism is relative to previous work for assessments.

Occurrence of polychlorinated biphenyls in Chelonia mydas from northeastern and southeastern coast of Brazil

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Polychlorinated biphenyls (PCBs) are persistent, toxic and widely distributed through the atmospheric transport and ocean currents. PCBs can mimic natural chemicals such as hormones and can disrupt the chemical processes of living organisms, some weaken the immune system. The lack of an efficient pathway for the degradation of these compounds, combined with their hydrophobicity, has led to its accumulation in various marine organisms and may undergo a process of bioaccumulation and biomagnification along the food chain. Among organisms more susceptible to the actions of organochlorines are sea turtles, especially species such as green sea turtle (Chelonia mydas), which has its occurrence associated with coastal regions, where the highest incidence and accumulation of contaminants occurs. The Chelonia mydas is the largest hard-shelled turtle, which is distributed throughout the oceans, in areas of tropical and subtropical waters. The present work aims to verify the occurrence of PCBs in the species Ch. mydas. Liver samples were collected juvenile dead turtles on the coast of Ubatuba (n=30), state of São Paulo and on the coast of Aracaju (n=40), state of Sergipe, located respectively in southeastern and northeastern region of Brazil. This study used data generated by the Regional Monitoring Program of Stranding and Abnormalities in the Caribbean, in collaboration with the Fundação Mamíferos/Aquaticos, FMA, in partnership with Tamar/CBM, as a mitigation measure requested by the Federal Environmental Licensig, conducted by CGPEG/IBAMA. The PCBs were extracted with organic solvents and the purification was made with concentrated acid. The identification and quantification of PCBs was performed in gas chromatograph with mass spectrometer. PCBs were found in 76% of samples from Ubatuba and 43% of Aracaju. Total PCBs of 17 congeners were found in concentrations of <0.38 to 17.4 ng g⁻¹ of wet weight in 25% and <0.38 to 142.6 ng g⁻¹ of wet weight in 25% of samples of Aracaju ranged from <0.38 to 142.6 ng g⁻¹. Predominance of light PCB congeners (3-5 chlorine) can be observed within the majority of the samples analyzed, showing similar distribution profile in the turtles of both areas that can be related with long range atmospheric transport. The accumulation of PCBs in marine organisms can be linked with omnipresent turtle diet during juvenile stage. More over the low levels are associated with the maturity of those turtles.

Investigation and development of a non-invasive LC-MS/MS quantitative method to determine the stress levels of whales

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The aim of this project is to determine if it is possible to detect the presence of hormones in whale blow samples, and see if the hormone levels could be related to stress due to their environmental situation. If successful the project would enable a non-invasive method to be developed to determine the stress levels of species that are difficult to study. Specific hormones, progesterone and cortisol, were monitored in samples of whale blow from a variety of different whale species. The results showed that both cortisol and progesterone were detected easily in the lowest calibration standard (0.5 pg/µL) suggesting lower detection limits are possible with further method development. Reproducibility was excellent, %RSD for both compounds were less than 2% for 2 replicates of a 5 pg/µL standard. Linearity for both compounds was excellent; r² = 0.999 for cortisol and r² > 0.999 for progesterone. Cortisol was detected in the majority (22/25) of samples at a level higher than the lowest calibration point. Progesterone was detected in 13 samples higher than the lowest calibration point. For all samples parallel collection of both MRM & full scan data was obtained and is shown for each of the species types. This set of data enabled other compounds to be identified and the use of this test organism is relative to previous work using corals. Research continues on the development of sub-lethal endpoints for assessment.

Measurement of trace element and perfluorinated compound (PFC) concentrations and species identification of shark fins from the Hong Kong market

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Shark populations are declining around the world, largely due to overfishing and the global shark fin trade. In contrast to many other marine predators, such as marine mammals, there is a lack of information on the contaminant status of sharks, likely because sharks are often more difficult to obtain. The large pelagic species, in particular, are often available only for opportunistic sampling as bycatch, but are well represented in the shark fin market. The purpose of this study was to assess the levels of two groups of trace elements and perfluorinated compounds (PFCs), in shark fins purchased from dried seafood shops in Hong Kong, one of the largest markets in the world for shark fins, and to identify the sampled species using DNA sequencing. Shark fins, composed primarily of collagen, can be used for monitoring protein-bound contaminants, and also can be used to assess the hazards of human exposure to these chemicals through the consumption of shark fin soup, a traditional Chinese dish. Shark fins, in the current study, were purchased and analyzed for trace element (Ag, As, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sh, Se, Ti, and Zn) levels using microwave digestion and inductively coupled plasma-mass spectrometry (ICP-MS). A new method was developed for the extraction of short- and long-chain PFCs from shark fins and PFC concentrations were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). DNA was extracted from the purchased fins using a commercial kit. Species were identified using validated primer sequences. Trace element concentrations varied among individuals and species, and hazard assessment of the measured levels indicated that the overall risk to human consumers was low. The results of the species identification confirmed the presence of shark species listed as threatened or endangered by the International Union for the Conservation of Nature on the Hong Kong market, and will be discussed in the context of changes in shark populations over the last 10 years, as well as the occurrence and bioaccumulation of trace elements and PFCs in sharks.

Persistent organic pollutants and heavy metals in marine species stranded, threatened of extinction

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Sea turtles and cetaceans are two groups of species with most of their members threatened or endangered in some measure and, therefore, protected. The current social and industrial development has produced new and persistent forms of pollution reaching coastal and marine waters and the ecosystems that these support. Currently, in the Member States of the European Union, the concentration of selected PAHs (Polycyclic Aromatic Hydrocarbons), pesticides, PCBs (polychlorinated biphenyls), PBDEs (polybrominated diphenyl ethers), etc. Globally, studies of contaminants in ceteceans and sea turtles stranded have been developed especially in the last ten years. However, still a considerable number of gaps in this field, and the need for more data on samples to obtain a less biased information. Studies have focused primarily on organochlorine and heavy metal, with very few or absent studies with other persistent organic pollutants. Due to both to the fact of location into the top links of the food chain (being some of them super-predators) and to the presence of large blubber layer, they tend to biomagnify and to bioaccumulate persistent organic compounds in the case of cetaceans of the lesser taxonomic degrees, although in both groups are both types of compounds, the concentration ranges are different. In this work, a review of studies is showed, focusing in the chemicals, levels, oral, and species.

Organic pollutants in tern eggs from Rodrigues Island - Indian Ocean

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Very few organic pollutants in birds occur in general, and marine birds in particular, from the Indian Ocean. The Indian Ocean has Africa to the east and Asia to north - areas where active use of POPs such as DDT is used for malaria control and where legacy POPs such as PCBs have only recently begun to be phased out. The Indian Ocean has many small islands where large marine bird colonies can be found; many of these islands are quite isolated.

The aim of this study was to investigate the potential of such isolated islands as global background sites of persistent organic pollutants. We collected 10 eggs each of the Common Noddy (Anous stolidus) and Sooty Tern (Onychoprion fuscatus) breeding colonies from Cocos Island (19 43S, 63 17E), a marine reserve near Rodrigues Island.
Roguies Island is part of the Republic of Mauritius, and has a small human population with no industry other than fishing and an oil-fired power plant. Pooled samples were analysed for 47 and -100 were detected. No further analyses of brominated compounds were done. All eggs were then analysed for HCB, PCB, DDT, HCH, mirex, chorine derivatives. Geometric means were (ug/g wet mass, in all cases, Common Noddly is listed first, flowed by Soory Tern): HCB 0.37 and 0.4; mires 0.92 and 0.66; total DDT 1.71 and 2.4; total chlordane 0.092 and 0.14; total PCBs 2.1 and 2.5; total toxaphenes 0.24 and 0.58; total HCH 0.23 and 0.22. Although the levels were very low and close to detection limits for some compounds, t-tests of log-transformed data revealed significant differences (p<0.05) only for mires, chlordane, and toxaphenes. The slight differences between the results indicate possible differences in feeding patterns. Since these species are not endangered and bred in large colonies, their eggs are ideal monitoring tools. Bird eggs from islands such as these and elsewhere in the Indian Ocean may be ideal candidates for monitoring the effectiveness of interferences on an ocean scale.

TU 449 Assessments of biotransformation and ecotoxicological effects of alkylated Polycyclic Aromatic Hydrocarbons
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Even though spiked oil has been a subject of examination for many decades, there still exist major unresolved gaps in the knowledge of the environmental fate and effects of oil compounds. Alkylated PAHs are much more abundant than regular PAHs. In spite of this and their known persistence and human carcinogenic effects they have only been given little attention regarding ecotoxicological impacts. This work focus on the environmental fate and effects of four methylated PAHs: 1-methylphenylene, 1,6-dimethylphenylene, 5-methylbenzpyrene and two regular PAHs: pyrene and phenanthrene. The assessment is divided into: a) determination of bioavailability and identification of biotransformation products and c) toxicity testing of the methylated and regular PAHs. The toxicity was determined using standard Daphnia magna immobilization tests. Nereis diversicolor was used as the biotransformer because this worm exists in large numbers, and accounts for a significantturnover of carbon in the marine seafloor. Identification and quantification of biotransformation products was performed using UHPLC connected in series to a fluorescence detector and a Quadrupole Time-of-Flight Mass Spectrometer. Our results show that:

a. Alkylated PAHs are biotransformed at a much higher rate than regular PAHs
b. Carboxylates are the most abundant transformation product of the alkylated PAHs, whereas no phase I products were found for regular PAHs

2-Crino phase II products (glucuronide, glucoside, sulfate- and glucose conjugates) are found in both types of compounds, however glucose conjugates are only found for alkylated PAHs.
d. It is the hypothesis that the toxicity tests show that the methylated PAHs are more toxic than the regular PAHs, however these tests have not been performed yet.

While a last biotransformation rate may seem as a good feature for the methylated PAHs, as they will be removed last, there is also evidence that biotransformation of PAHs may produce compounds that are more toxic than the PAH itself. Hence, a high biotransformation rate may increase the environmental concentration and thus the exposure concentration of toxic transformation products. The high production of carboxylates signify the need for further ecotoxicological assessments of these compounds, as they are produced in high quantity and at a relatively high rate. Furthermore, these products are water soluble and will therefore be transferred to the water column.

TU 450 Addressing emerging issues in marine ecotoxicology with novel analytical techniques
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Two emerging issues in marine ecotoxicology are (1) the relative importance of animal physiology (elemental homeostasis) towards metal toxicity in transitional waters with intermediate salinities and (2) metal mixture toxicity. For both issues a mechanistic understanding of metal and element homeostasis upon mixture exposure is needed to enhance fundamental knowledge and applied risk assessment procedures. This study aimed at investigating the physiological impact of metal (mixture) toxicity on the homeostasis of mussels. We used micro-X-ray fluorescence (XRF) techniques to analyse mussel gills.

Gills of mussels (Mytilus edulis) from a coastal area of approximately 1.5 cm, exposed to a control or copper contaminated artificial seawater, were dissected and (a) immediately cryogenically frozen on-site or (b) dehydrated in a graded acetone/water series with hexamethyldisilazane (HMDS). Elemental distributions were analysed with synchrotron-based micro-XRF. The gills were first scanned with x-rays at different energy levels and Cu concentrations, upon which HMDS-embedded gills were analysed with laboratory source based micro-XRF. In a proof-of-principle experiment to study metal mixture toxicity, small M. edulis were exposed for 48 h to one of the following treatments in artificial seawater (i) control, (ii) 100 µg/l added Cu, (iii) 1 µg/l added Zn or (iv) a mixture of 100 µg/l Cu and 1 µg/l Zn. Again, HMDS-embedded gills were analysed with micro-XRF.

Preliminary results indicate that the internal distribution of metals in mussel gills at the micro scale is not significantly affected by the dehydration and HMDS fixation and enhanced tissue preservation, compared to the cryogenically prepared samples. Gills of mussels exposed to 40 µg/l Cu at a salinity of 32 psu exhibited a decrease in K content and an increase in Cu and Zn content. Preliminary results indicate that the internal distribution of metals in mussel gills at the micro scale is not significantly affected by the dehydration and HMDS fixation and enhanced tissue preservation, compared to the cryogenically prepared samples. Gills of mussels exposed to 40 µg/l Cu at a salinity of 32 psu exhibited a decrease in K content and an increase in Cu and Zn content. Again, HMDS-embedded gills were analysed with micro-XRF. There was a decrease in K content and an increase in Cu and Zn content. The results indicate that the increased tissue fixation is not significantly affected by the dehydration and HMDS fixation and enhanced tissue preservation, compared to the cryogenically prepared samples. Gills of mussels exposed to 40 µg/l Cu at a salinity of 32 psu exhibited a decrease in K content and an increase in Cu and Zn content. Again, HMDS-embedded gills were analysed with micro-XRF. The results indicate that the increased tissue fixation is not significantly affected by the dehydration and HMDS fixation and enhanced tissue preservation, compared to the cryogenically prepared samples. Gills of mussels exposed to 40 µg/l Cu at a salinity of 32 psu exhibited a decrease in K content and an increase in Cu and Zn content.

Micro-XRF can provide fundamental mechanistic knowledge about physiological effects of metal mixtures or metal toxicity in transitional waters. It revealed different physiological consequences of metal mixture exposures compared to single metal exposures.

TU 451 Salting-out effect in aqueous NaCl solutions- experimental data and modeling
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Increasing NaCl concentrations in aqueous solutions shift the equilibrium partitioning of organic solutes towards non-aqueous phases like organic matter or air. This so-called salting-out effect has long been known but there are rather few experimental data and many of these data are contradicting. Here we present experimental data on the salting out effect of NaCl on a large and diverse set of organic chemicals together with an evaluation of various modeling approaches.

TU 453 Photobromination of phenol and salicylic acid in coastal marine waters
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A large number of experimental studies aiming at understanding the photo-fate of organic pollutants in fresh surface water have been published. In contrast, considerable less attention has been paid to the possible environmental significance of photoreactions of anthropogenic compounds in coastal seawater. The formation of halogenated organic chemicals occupies a special position since they have complex diversities of biologic effects: Immunotoxicity, endocrine toxicity, reproductive toxicity, carcinogenesis. The biotic processes are the main published and focused natural origins of halogenated organic chemicals while to date relatively little attention has been focused on abiotic processes. The possible sources of biotic chlorination and bromination are: UV induced direct release of CO2 or UV induced direct formation of hydroxyl radicals, chlorine species, and Br2 radicals as a consequence of the reaction between hydroxyl radicals and Cl- or Br- species. However, the quantitative importance of these sources are not fully assessed yet and probably do not account for the total halogen formation of halogenated derivatives in seawater. This work presents results of photolysis studies in coastal seawater, using phenol and salicylic acid as model molecules. Natural seawater was sampled in the Gulf of Lyon (southern France), a coastal area with depth around 10-20 m, characterized by a moderate hydrological recycle, and subjected to noticeable anthropogenic inputs, from the city of Nice. Twenty-five hours after photon exposure. Phenol and salicylic acid concentrations were adjusted to levels (c) of 50 µg/l under natural light and (c) of 10 µg/l under simulated solar light in presence of different solar generators (i.e. superoxide and nitric oxide). The extension of indirect photolysis and the formed intermediates were determined. The formation of halogenated intermediates might originate from the reactivity of hydrobromic acid (HBrO) resulting from the oxidation of bromine ion by peroxynitrite (ONOO-) and should be catalysed by bromamines. A potential photochemical source of trihalomethane in coastal seawater will be discussed.

TU 454 Laboratory scale simulation of CO2 leakages from stable marine geological storage: metal accumulation and biological effects on the benthic organism Rudipatapipharumphilippines
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Nowadays, the injection and storage of CO2 in marine geological formations is proposed as one of the potential strategies to decrease the atmospheric CO2 concentrations in order to avoid the abrupt and irreversible repercussions of climate change. Nevertheless, practical implementation of this technical option could produce significant impacts on marine ecosystems due to possible CO2 leakages that may occur during the injection and sequestration procedure. This work investigates the possible effects of variation in sediment pH derived from CO2 leakages on metal bioaccumulation. To this end, a lab scale experiment involving direct release of CO2 was conducted using the model benthic organism, Rudipatapipharumphilippines. Bivalves were exposed during 10 days to sediment samples under established pH conditions (8.0-8.6). Survival, burrowing activity and metal accumulation (Fe, Co, Pb, Mn, Al, Zn, Cu, Hg and As) on whole body were employed as endpoints. After 10 days of exposure, 100% mortality was observed at pH 6.0. Non significant differences for mortality rate were found in 7.0 and 6.5 treatments with regard to the control (7.9). Burrowing activity was affected by pH, decreasing the activity at low pH levels. In general, metal concentrations in clam tissues were affected by pH. These results...
conclude that CO2 leakages might provoke an increasing of sediment toxicity linked to metal mobilisation.

TU 454  
New piling materials for seawater environments: an ecotoxicological assessment  
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Sediments are brought into contact with seawater contaminated with aluminium either by using aluminium sacrificial anodes in a thermostated cell with a classical three electrodes system or by dissolving an aluminium salt: aluminium sulphate (Al2(SO4)3,18H2O) or aluminium chloride (AlCl3, 6H2O) . In order to evaluate the potential aluminium mobility and the evolution of its mineralogical speciation, single and sequential extractions (2) were applied to natural sediments.

In order to evaluate the potential aluminium mobility and the evolution of its mineralogical speciation, single and sequential extractions (2) were applied to natural sediments and clays before and after aluminium contamination. All the leachates are analysed by ICP-AES (Varian, Vista MPX).

The results show that whatever the mode of contamination was, the additional aluminium is more labile than aluminium present initially in sediments and clays.

TU 455  
Mobility and mineralogical speciation of aluminium in contaminated sediments and clays  
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Aluminium sacrificial anodes are commonly used for the protection of metallic structures in marine harbours. The environmental impact of these anodes remains poorly understood. Indicators of aluminium toxicity in marine organisms have been linked to the increase of aluminium concentration in water and finally in sediments (1).

The aim of this study is to investigate the mineralogical speciation of the solubilized aluminium in sediments and clays.

Results show that whatever the mode of contamination was, the additional aluminium is more labile than aluminium present initially in sediments and clays.

TU 456  
Identification of contaminants in marine waters using high resolution fractionation combined with mass spectrometry  
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4Primary production by microalgae embodies the carrying capacity of marine ecosystems and is primarily linked to nutrient availability and light. However, recent studies indicate that certain anthropogenic contaminants and natural toxins may have a direct impact on coastal planktonic communities and hence on the carrying capacity of estuarine and marine ecosystems. How marine ecosystems respond to and mix effects of anthropogenic compounds and natural toxins under field conditions. Linking biological responses and chemical compounds is often problematic due to the large numbers of different compounds present in marine waters. In our study we aim to identify the contaminants that are responsible for toxic effects on photosystem II efficiency in microalgae using GC-MS and LC-MS. We use Effect Directed Analysis (EDA) to determine the compounds that have an effect on growth in microalgae in order to quantify the toxic pressure in Dutch estuaries and coastal waters. In EDA, analytical chemistry is combined with bioassay analysis to isolate and ultimately identify the contaminants in a complex sample that are responsible for the observed effects. We use passive samplers to extract contaminants from estuarine/marine waters during a period of 6 weeks. By using ultra performance liquid chromatography with UV detection, extracts are fractionated into 96 wells plates, resulting in far more, smaller fractions per sample than commonly used in EDA. After evaporation of the solvent the well plates can be directly used for microalgae toxicity assessment using Pulse Amplitude Modulated fluorescence (PAM). With this high resolution fractionation technique the complexity of the fractions is reduced to simplify the chemical identification of the contaminants responsible for the biological effect.

TU 458  
Human health risk assessment of selected metals in the diet of the UAE community  
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TU 458  
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Stockholm University, Stockholm, Sweden  
In recent years halogenated phenolic compounds (HPCs) such as hydroxylated polybrominated diphenyl ethers (OH-PBDEs) have been found in many species from the Baltic Sea, e.g. cyanobacteria, red alga, blue mussels and a variety of fish species (1, 2). Although these substances seem to be primarily natural products (3) they can also be released from waste incineration or other sources such as fire retardants (4, red alga) and marine plants. The effect profile of this mixture depends on environmental factors like temperature, light, wind or nutrient supply. Variations, e.g. due to climate changes might increase the levels of OH-PBDEs produced dramatically (5). Recent studies have shown that one of the most ubiquitous OH-PBDE found in marine fish, 6-OH-BDE 47, is toxic to zebrafish Danio rerio embryos (6). This developmental toxicity is, at least in part, due to potent inhibition of mitochondrial oxidative phosphorylation (OXPHOS), thereby disrupting the main source of cellular energy. In this study, we hypothesize that other HPCs may also modify energy metabolism and that this effect might explain some of the severe health effects observed in Baltic Sea wildlife, such as wasting syndrome, decrease in fat content and reproductive insufficiency. Several HPCs found in different fish species in the Baltic Sea have been identified and synthesized and are currently being tested for effects on OXPHOS, with both in vitro and in vivo models. We have developed a novel method for monitoring the disruption of OXPHOS in vivo in a small fish species (Danio Rerio). The effects of HPCs on mitochondrial respiration in the living organism are measured using various techniques, including oxygen consumption, acid production and mitochondrial membrane potential. Preliminary results indicate that a number of HPCs disrupt OXPHOS. Studies are currently ongoing to link these new effects on energy metabolism in the zebrafish embryo with effects, like a decreased fat content, found in fish sampled from the Baltic Sea. These results are of general importance as fish are widely consumed by humans and other organisms. In this study, we hypothesize that other HPCs may also modify energy metabolism and that this effect might explain some of the severe health effects observed in Baltic Sea wildlife, such as wasting syndrome, decrease in fat content and reproductive insufficiency. Several HPCs found in different fish species in the Baltic Sea have been identified and synthesized and are currently being tested for effects on OXPHOS, with both in vitro and in vivo models. We have developed a novel method for monitoring the disruption of OXPHOS in vivo in a small fish species (Danio Rerio). 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References
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TU 460  
The challenge of sustainability of marine resources: results of FAROS Initiative  
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Discrepant results are discussed by the FAO as “that portion of the total organic material of animal origin in the catch, which is thrown away, or dumped at sea for whatever reason. It does not include plant materials and post harvest waste such as offal. The discards may be dead, or alive”, i.e. both no commercial as well as target species. No commercial species are generally dumped because it is undesirable (i.e. there is no market or other use for it) and therefore uneconomic to keep on board. Target species may be discarded due to poor economic conditions (below Minimum Landing Size) of the species or because of resource overexploitation. Nowadays, there is a common and positive agreement (among citizens, NGOs, the fishing sector, policymakers, scientist, etc.) that perceives discards as very negative and that solutions have to be implemented. In this framework of promoting the responsible and sustainable management of the European fishing activity, the European Commission developed a number of actions directed to the development of policies “to reduce unwanted by-catches and eliminate discards in European fisheries”, as well as to make “the best possible use of the captured resources avoiding its waste”. In this framework of defining strategies and solutions to accomplish with the EU policy of “no discards”, the
Among the broad spectra of pollutants reaching the ecosystems, Polycyclic Aromatic Hydrocarbons (PAHs) are of special interest. Introduced in remote oceanic regions by this metal can occur from several sources including after its use as biocide. Here, the effects of this metal on interspecific competition were investigated using planktonic organisms of standing waters.

We will show a framework of TKTD models, IBM models and an aquatic ecosystem model for quantifying responses to stress at various levels of ecological complexity for such as time to recovery, extinction probability, or mean population density.

In this presentation, we will introduce a concept to integrate the TKTD and IBM models in an ecosystem model framework, which on the one hand allows to consider new forcing functions for the physical condition of ponds and lakes are also included in this modelling concept.

ET414P - Bringing ecological processes into ecotoxicological risk assessment

Mercury is considered one of the most toxic metals and a high concern over its toxic effects on organisms, populations and ecosystems exist. Contamination of aquatic ecosystems by this metal can occur from several sources including after its use as biocide. It has been observed that zooplankton can be more sensitive to these pollutants than phytoplankton, the balance between growth and losses of phytoplankton populations independently showed a variety of responses and sensitivities, being in some cases phytoplankton populations more resistant to PAHs. However, their interactions have been scarcely analyzed until now.

Recently, new approaches of combining toxicokinetic-toxicodynamic (TKTD) models and individual-based population models (IBM) are being developed to simulate effects of chemical exposure on the population level of sensitive organisms. These individual-based population models provide applicable endpoints for the ecological risk assessment such as population extinction probability, or mean population density.

In this presentation, we will introduce a concept to integrate the TKTD and IBM models in an ecosystem model framework, which on the one hand allows to consider new more ecological relevant endpoints on the ecosystem level in the risk assessment (which is also of interest in the context of ecosystem services), and on the other hand enables to cover a broad and realistic range of environmental conditions, as well as variable weather conditions and exposure patterns as important forcing functions for the physical condition of ponds and lakes are also included in this modelling concept.

By using this model concept indirect effects can emerge on both, population and ecosystem level: indirect effects on the biomass of planktonic organisms due to competition and predation dependent on the implementation of food web, as well as indirect effects on water quality parameters such as oxygen concentrations, salinity, temperature and water turbidity. These indirect effects have to be addressed in higher tier studies. For example, time to recovery of a treated planktonic population may vary due to indirect effects, e.g. increasing resources, decreasing competition and predation, respectively.

We will show a framework of TKTD models, IBM models and an aquatic ecosystem model for quantifying responses to stress at various levels of ecological complexity for planktonic organisms of standing waters.

TU463 Understanding the influence of competition on effect and recovery of Daphnia spp. from pesticide exposure in outdoor microcosms

TU462 Aquatic ecosystem model framework for use in refined environmental risk assessment

TU461 Effects of polycyclic aromatic hydrocarbons on growth and grazing rates of Atlantic Ocean Phytoplankton communities

TU458 Effects of mercury on biomarkers of Corbicula fluminea, a non-native invasive species in Europe

Project FAROS - co-funded under the LIFE+ Environment Program of the European Union - takes advantage of the background of experience, good practices and previous technologies to give the guidelines to define a global and real-time information system both on board and in land. This system will be the core of an efficient and optimal discard management network of the actors involved in the fishing activity (fishermen and fleets, ports, industries, etc.) by exploiting of the existing synergies between them in order to achieve the minimalization of discards/catch as well as their optimal valorization to recover and to produce valuable chemicals of interest in the food and pharmaceutical industries.

TU460 Brominated flame retardants and dechloranes in eels from German rivers

TU456 Effects of esfenvalerate on Daphnia magna populations in outdoor pond microcosms
Fluxes were less sensitive to mercury than its native competitors. Furthermore, the findings indicate that contamination of aquatic ecosystems by mercury may act in favor of non-native invasive species in situations of competition with native ones. This study was carried out in the scope of the project “NISTRACKS - Processes influencing the invasive behaviour of the non indigenous species Corbicula fluminea (Mollusca: Bivalvia) in estuaries” identification of genetic and environmental key factors” funded by the Portuguese Foundation for the Science and the Technology (FCT) (PTDC/AAC-AMB/102121/2008) and FEDER COMPetes fund (FCOMP-01-0124-FEDER-000856). P. Oliveira had a PhD grant from FCT (SFRH/BD/82402/2011) funded by national funds of the MCTES and FEDER European funds (POPH-QREN-Technology 4.2.).

TU 465 Predation: boost or buffer of chemical effects? 
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Current experimental approaches to establish chemical effects on natural communities are fundamentally limited by the requirement of standardization and long-term experiments. To this end, the benthic diatom Seminavis robusta was used to assess the effects of chemicals on biodiversity and function. We studied the effects of five hydrophobic pesticides (atrazine, isoproturon, flumioxazin, fludioxonil, and linuron) on the cell density and chlorophyll a concentration measured by inverted microscopy and the photosynthetic efficiency, as determined by Pulse-Amplitude-Modulation (PAM) fluorometry, respectively. Treatment concentrations were 0, 20, 100 μg/l for each compound. In order to assess the trophic interactions, several level of food availability and competition were investigated. We focused on the sensitivity of the entire community and its components rather than to the single species level. We hypothesised that the chemical effects will be lower in systems that include size-selective predators. As a consequence, extrapolation of effects from those systems will underestimated population effects in systems without or with predation limited systems.

TU 466 Inferring the effects of chemicals on biodiversity: a case study with the herbicide linuron
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Ecotoxicological experiments are typically performed under standard conditions to reduce uncertainties and to allow standard comparisons between studies. However, standard conditions can be unrepresentative for the field as natural events (e.g. seasonality) and anthropogenic contributions (e.g. carbon emissions) continuously induce shifts in environmental conditions. In the current study, we examined to what extent the sensitivity of the benthic diatom Deminavis robusta to the herbicide atrazine, a commonly used photosynthetic inhibitor, is changed after addition of a carbon resource. To this end, the benthic diatom S. robusta was treated with 0, 20 and 100 μg/l atrazine in 20 ml microcosms with f2 medium (artificial seawater with nutrients) for 5 days (0.472) .

TU 467 Increasing resource availability compensates atrazine effects on the benthic diatom Seminavis robusta
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The density of the S. robusta population dynamics and that allows for the systematic exploration of predator type as well as kairomone effects. Our study reveals that in case of a chemical that mainly affects small individuals, direct and indirect effects of gap-limited predation favour size classes that are more vulnerable to chemical exposure. In this case, the release of a chemical would co-evolve with prey and predator life stages. As contrast, chemical effects will be lower in systems that include size-selective predators. As a consequence, extrapolation of effects from those systems will underestimated population effects in systems without or with predation limited systems.

TU 468 Environmental context determines community sensitivity of freshwater zooplankton to a pesticide
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The environment is currently changing worldwide, and ecosystems are being exposed to multiple anthropogenic pressures. Understanding and consideration of such environmental conditions is required in ecological risk assessment of toxicants, but it remains basically limited. In the present study, we aimed to determine how and to what extent alterations in the abiotic and biotic environmental conditions can alter the sensitivity of a community to an insecticide, as well as its recovery after contamination. We conducted an outdoor microcosm experiment where in zooplankton communities were exposed to the insecticide esfenvalerate (0.03, 0.3, and 3 μg/L) under different regimes of solar radiation and community density, which were expressed as terms of food availability and competition. In addition, we measured, using multivariate statistical methods, such as principal response curve analysis, the effects of both radiation and community density on the community. We focused on the sensitivity of the whole community and related it to the sensitivity of each species in the community. We found that community sensitivity varied markedly depending on the environmental conditions, which represented different levels of food availability and competition. We focused on the sensitivity of the entire community and its components rather than to the single species level. We hypothesised that the chemical effects will be lower in systems that include size-selective predators. As a consequence, extrapolation of effects from those systems will underestimated population effects in systems without or with predation limited systems.

TU 469 Pesticide stress in mountain streams: manipulative experiments and metrics strategies
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Pesticide loads in streams are potentially one of the most relevant stressor for macroinvertebrates communities. Nevertheless real effects provoked at the community level are still poorly unknown. To increase our knowledge of effects at this level of organization, descriptive approaches of natural systems are useful but not sufficient, since huge natural and anthropogenic variability may act as noise, causing the power of the investigation to drop. Biomonitoring should be coupled with manipulative experiments able to establish causal relationships between a pesticide stressor and the observed effects. Furthermore, to get a deeper knowledge of complex systems, it is necessary to make experiments which reflect this high degree of complexity.
The stream mesocosm consisted of 12 artificial channels and was operated as a flow-through system, relying on water pumped from a diversion channel of the Llobregat River (Barcelona, Spain). Different concentrations of a salt saturated solution were released in pulses from a 2000 l tank using an irrigation system. River cobbles were colonized with aquatic macroinvertebrates (predatory mites, Gammarus, Asellus aquaticus) and the colonization of both the cobbles, invertebrates’ drift and algal biomass were collected. Leaf consumption rates were measured in order to explore possible sub-lethal effects of the salt spikes. The pollution community colonizing the leaves was also characterized. Preliminary results show that the salt treatments caused a decrease in the invertebrate densities, a higher invertebrate mortality and a decay of the leaf consumption rates. The salt spikes prevented algal growth, but no effect was registered for the fungal communities.

TU 470

Evaluating recovery following ecotoxicological effects: a general approach. (The “colonization concentration” - CCy) by considering that CCy = AC(100 - x).

To validate this “colonization concentration” concept we used the earthworm E. andrei as a model organism and copper as a model chemical in a 3 step process: (1) calculate AC values and confirm that the avoidance test using a dual test chamber and how it is currently performed (placing the animals in the middle line) gives the same results when compared to the original method (under the control, i.e., using a 3 compartment design - escape response); (2) estimate the number of individuals expected in the compartment with soil contaminated with the CC50 is not dependent of their previous exposure to the contaminant (i.e., passing through a contamination phase); (3) get empirical data proving the concept over a larger scale by simulating a contamination gradient.

Effects of sub-lethal pesticide exposure on movement behaviour of Asellus aquaticus

Joseph C. Alknis, Brian D. Smith

We investigated the influence of pesticide exposure, habitat quality and density on the movement of Asellus and Gammarus, we performed video tracking experiments to derive information on movement behaviour of individual A. aquaticus under different conditions. The experiments testified for the effect of exposure to two different neuro-toxic pesticides (chlorpyrifos and imidacloprid) at different concentrations with varying population densities in the experimental setup. An additional test studied the impact of environmental heterogeneity, by using information of one of the species present (A. aquaticus) when not exposed to the other. We found that study different population densities and habitat qualities (e.g. presence of food or shelter) since different locomotion activities were assumed under varying conditions.

TU 471

Life history traits as predictive parameters for recovery

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Ecological recovery is gaining importance in both prospective (e.g., pesticides) and retrospective (site specific) ecological risk assessment. Thus, modelling recovery, and associated recolonisation, is of paramount importance to better define levels of protection for the soil system. The recolonization process of contaminated soils is conditioned not only by the existence of suitable habitat conditions (including the degree of contamination) but also by the existence of a soil community acting as an external source of individuals. Moreover, their ability to detect the contaminants and to move towards contaminated patches is a key issue.

Soil avoidance tests are widely used and give us information about the ability of organisms to detect the contaminants and, more important, the percentage of the population that is able to avoid it (not able to colonize the contaminated soil). In this context, avoidance data may also provide information on the potential for recovery of soil fauna at contaminated sites. So, a concentration causing a certain percentage of avoidance (ACx) can be used to derive a concentration originating a certain percentage of colonization (the “colonization concentration” - CCy) by considering that CCy = AC(100 - x).
the efficacy of the remediation measures already implemented in the Paramos lagoon, by comparing the toxicity of surface versus subsurface sediments, as depth profiles in the tested cases. The potential for using methemoglobin within Chironomus larvae as a biomarker for nitrates in water and hence risks of methemoglobinemia among children, pregnant women, and breast-feeding women. The nitrate levels in water ranged from 0.5 mg/l to 30.0 mg/l NO₃-N with a mean of 5.7 mg/l NO₃-N.

TU 475
A comparison of the chronic toxicity of lead between laboratory and field populations of the great pond snail (Lymnaea stagnalis) C. Nys, C.R. Janssen, K.A.C. De Schamphelaere
University of Ghent, Gent, Belgium

Recent research demonstrated that Lymnaea stagnalis is among the most sensitive aquatic organisms for lead. However L stagnalis and other species of the Lymnaeidae genus occur in the majority of aquatic systems across Europe, even in polluted water bodies. It was therefore hypothesized that L. stagnalis may be more sensitive in the lab than in the field. Indeed the high sensitivity of L. stagnalis could be the result of the tendency of laboratory populations to show a higher level of inbreeding and a reduced genetic variability compared to field populations. A reduced genetic variation can lead to a higher sensitivity towards pollutants. We compared the chronic toxicity of lead between three laboratory and nine field populations. The total population of the two sites, the one located close to a contaminated tributary and the other close to the centre of the lagoon, were those with lower organism responses and higher uncertainties in what regards risk. Also, for all except one site (one of the most contaminated), organism performances were generally better with surface populations. Although the selected test battery proved to have potential to map toxicity levels within the Paramos lagoon and the results were reanalyzed according to the test toxicity assessment (e.g. additional tests and sites, chemical analysis).

TU 476
Use of indicators of toxicity and biological communities as explanatory variables and responses in ecological risk assessment of an aquatic environment K.T. Tallini
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This study used toxicity tests with Hyalella azteca and Ceriodaphnia dubia and biological indicators of zooplankton and benthic communities as part of a model of ecological risk assessment applied to a stretch of the Rio Jacui-RS. The methodology was considered as an explanatory variable in this study, the evidence for the abiotic compartments, surface water and sediment. The Toxicity Indicator was generated from the use of toxicity tests with Hyalella azteca and Ceriodaphnia dubia which allowed to assess the ecotoxicological evidence from the results of acute effect, chronic effect or no effect when compared with the proper local standards of environmental quality in Brazil, so the CONAMA Resolution 245/2005 establishes the criteria for surface water quality classes 1 and 2 should not have chronic toxic effects and that water class 3 and 4 should not show acute toxic effect. CONAMA Resolution 343/2004 establishes the criteria for classification of sediments to be dredged two quality levels, a level which provides the low probability of adverse effects to biota and level 2 which provides a likely adverse effect on biota. For the evaluation of biological evidence has been proposed the use of a Biological Indicator for each sample point data were used in species richness, diversity index of Shannon-Wiener and Pielou's evenness of the number density of the species of zooplankton and benthic communities monitored. The four measures of biological attributes were transformed into proportions of one to zero by dividing their highest value obtained from the sample points. For the expression of the Biological Indicator used the number of species to the rayes obtained per each sample point. As a result the Cho Index revealed acute toxicity for all sampled points. Biological Indicator Results were expressed as a numeric attribute ranging from 0 to 4. Within this range the lowest value found for the zooplankton community was the average of the highest P4 and P2 the average. The benthic community presented as the average of the lowest and the highest value P3 average of P1.

TU 477
Perinereis gualpensis (Polyhecta: Nereidae) a suitable specie for biomonitoring south central Chilean estuaries M.J. Diaz-Jaramillo, R. Barra
EULA-Chile Environmental Sciences Centre, Concepcion, Concepción, Chile

Perinereis gualpensis is a key estuarine specie from Chilean pacific coast estuaries because it is a sandy mudd sediments are predominant. Comparing contaminant concentrations, the responses at different levels of organization in sediment, in situ and laboratory approaches indicates the suitability of P. gualpensis for future biomonitoring programs. On this basis the obtained results indicate: (1) site-specific responses and fast uptake of Hg from polluted sediments confirm the use of this specie as a bioindicator; (2) the ability to reflect anthropogenic changes through oxidative stress and antioxidant responses and other biochemical responses like Na+-K+-ATPase activity showed a higher percentage of acute toxicity for all sampled points. Biological Indicator Results were expressed as a numeric attribute ranging from 0 to 4. Within this range the lowest value found for the zooplankton community was the average of the highest P4 and P2 the average. The benthic community presented as the average of the lowest and the highest value P3 average of P1.

TU 478
Application of Methemoglobin formation in Chironomus larvae to assess risks to nitrates in a poor urban neighborhood, in Eldoret, Kenya A.K. Kachere, O. Osano, J. Wakhusi, P. Raburu
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Application of short chain perfluorinated compounds (PFCAs) and perfluorinated sulfonic acids (PFSA) as well as some precursors were spiked together with a tracer in the supernatant of a water-saturated sediment column (length 1 m). The column was filled with coarse-grained medium sand and was fed with surface water (pH 7.8) from a surrounding pond. Water samples were collected from the supernatant and after 40 and 80 cm of sediment passage in regular intervals. Samples were extracted using solid phase extraction and LC-ESI/MS/MS was used for instrumental analysis. Concentrations in water samples were corrected with blank concentrations and with recoveries of massbalanced internal standards.

Results
Following concentrations of analytes in the supernatant ranged from 1.0 to 15.9 ng L⁻¹. Some precursors were not detected in any samples. Even fewer analytes were detected in 40 cm depth indicating occurrence of loss, presumably due to sorption to sediment. C₄ and C₆ PFCAs and PFSA showed tracer-like breakthrough in 40 cm and were slightly retarded in 80 cm depth. C₆ and C₈ PFCAs were detected in much lower concentration than short chain PFCAs and PFSA and were retarded by the sediment. 

Conclusions and Outlook
While short chain PFCAs and PFSA were not attenuated by the sediment longer chain ones showed retardation. In the next step the recoveries of the different PFCAs and
Four time-independent repetitions were carried out for each pH value. The maize was grown in four containers of 40 L nutrient solution under controlled conditions (pH-stat system). Once the pH was adjusted individual PFC substances were added to obtain a concentration of 100 µg L⁻¹ root medium. The PFC mixture was made up using liquid chromatography coupled to tandem or high resolution mass spectrometry with instrumental detection limits in the low femtogram range. An extraction, ion-pair extraction, solid phase extraction and dispersive clean-up on graphitized carbon. Final separation and quantification of PFSAs and PFCAs was performed using liquid chromatography with tandem mass spectrometric detection. The aim was to identify and quantify the major fluxes of PFAAs to and from the Baltic Sea. A mass balance was attempted for perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). A comparison of the estimated dietary intake relative to other exposure pathways indicates that approximately 70% of the total exposure to PFOA and PFOS is coming from the diet for the Swedish population. Estimated dietary intakes are used as inputs in the PBPK model and it is shown that model estimated serum concentrations of PFOA and PFOS compare favourably to measured serum levels from the year 2010, suggesting that dietary intakes are now approximately at steady-state with human serum levels of PFOA and PFOS will not stop declining and level off in the near future.
input pathways. Country specific WWTP emission data on a per capita basis from Sweden, Finland, Estonia, Latvia, Lithuania, Poland and Denmark were used to estimate the total WWTP effluent flux of PFAs from 55 of the largest cities along the Baltic coastline. PFNA fluxes from 15 of the major rivers discharging into the Baltic were compiled. Inputs through wet deposition directly into the Baltic Sea and inflow through the Danish Straits were calculated. As output pathway the outflow through the Danish Straits was estimated. Sediment burial represented by particulate organic carbon sedimentation was considered as a sink of PFAs.

The results show that direct wet deposition into the Baltic Sea and inflowing rivers are the two major input pathways of PFHxA, PFOA and PFOS, accounting for the largest part of the perfluorinated compounds (PFCs) effluents along the coastline. Thereby, the WWTPs along the coastline only contributed with a minor fraction to the total amount of PFAs discharged into the Sea. The net flow through the Danish Straits was estimated to be the major flux of PFAs out of the Baltic. Sediment burial was only a non-significant loss mechanism for PFAs. A sub-study of the river Oder catchment area showed that wet deposition with its runoff into the river was the major input of PFOA and PFOS also to this riverine system, whereas all effluents from WWTPs in the catchment area contributed much less PFAs to the Oder. This indicates that wet deposition is the overall most relevant input pathway for PFHxA, PFOA and PFOS to the Baltic environment. Taken together, the sum of all PFAs fluxes to the Baltic Sea exceeded the sum of outflows, suggesting that the PFAs levels in the Baltic Sea are still increasing today.

**TUPC1-7**

**Perfluorinated compounds in effluents from the largest US wastewater treatment plants**

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Studies conducted to date suggest that wastewater treatment plants (WWTPs) are one of the primary sources of perfluorinated compounds (PFCs) entering the aquatic environment, and that the concentration of PFCs in wastewater effluents typically exceeds that in surface water, ground water, or drinking water. This has raised concerns about human or environmental impacts among the public, media, wastewater facility operators, regulators, and legislators. However, data on the occurrence of PFCs in wastewater effluents in the US are scattered, due to the logistic challenges and analytical difficulties that conducting a nationally representative survey.

Our current work aimed to: a) assess the presence of PFCS and other chemicals of emerging concern in wastewater effluents in a nation-wide monitoring campaign, as well as evaluate their oestrogenicity, and b) provide an insight on the chemicals potentially reaching the US receiving waters. In this study, the 50 very large municipal WWTPs in the US were identified from the 2004 Clean Watershed Needs Survey. These plants produce about 17% of all the municipal wastewater discharge in the US, serving 20% of the US population. A 24-hour composite sample (total of 8 liters) of finished effluent from each plant were collected by EPA Regional or selected plant personnel, and then shipped to the EPA laboratory. For each effluent sample, 50 high priority pharmaceuticals, steroid hormones, alkylphenolic compounds, Bisphenol A and PFCs were measured along with the oestrogenic potency in a fathead minnow vitellogenin induction assay.

This presentation will focus on the results from the PFCs analysis. PFCs were detected in 100% plants, with C4-10 perfluorocarboxylic acids and C4, C6 and C8 sulfonates being detected in more than 80% of the plants. PFHxA, PFOA and PFOS were the predominant PFCs. Most of the effluents had comparable concentrations of PFCs to those previously reported, with median levels ranging between 10 and 30 ng/L. However, some effluents showed elevated concentrations (e.g. 880 ng/L of PFOS). Some plants sampled multiple times revealed large intra-day variability in the concentration of the selected PFC. There was no significant correlation found between PFCs concentrations and season/total discharge. Additionally, the effect of treatment characteristics of the selected plants on PFCs concentrations will be discussed in the presentation.

**TUPC1-8**

Are perfluorinated compounds (PFCs) subject to atmospheric transport and deposition in the high Alpine environment?

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Perfluorinated (PFCs) are of growing concern as global contaminants. They have been detected in biotic and abiotic matrices in both source and remote regions (e.g. Arctic). How they reach such remote environments is still under debate. One of the hypotheses for explaining their environmental fate is that their precursors are subject to long range atmospheric transport (LRAT) from industrialized source regions. For testing this hypothesis, a comparison with well known contaminants, such as POPs was made. POPs are semivolatile substances that can accumulate in cold environments in high latitudes, but also in areas of high altitudelike the European Alps. The "cold condensation" effect - the temperature driven shift in equilibrium partitioning towards sorbed phases - results in the accumulation of these chemicals in snow and ice, for example. During periods of thaw, the compounds can be found in the melt water of glacial streams.

This LRAT hypothesis for PFCs was tested in the Alpine environment in Northern Italy, in two alitudinal transects from glacier or mountain snow, to glacial streams and then to lakes. Snow and water were sampled in summer 2008 from two glacier (Lai and Fornti valleys) and then along the catchment to two of the major rivers in Italy: the Po and the Adda. Up to 24 PFC compounds were analyzed with HPLC-MS/MS, comprising perfluorooctylsulfonic acids, perfluorocarboxylic acids, perfluoro sulfonamides and perfluoro sulfonamidothanols. The C4-C8 sulfonic and C4-C12 carboxylic acids, as well as perfluorooctanesulfonamide (FOSA) were detected in the samples. Generally, higher concentrations of the perfluorooctyl acids (PFOA) were observed in the valley rivers compared to the higher altitude streams, highlighting the influence of point sources further down the catchment. However, PFOA were detected in the higher altitude streams with concentrations of the perfluorocarboxylates (notably PFBA, PFOA, PFNA and PFUnDA) higher in glacier/snow compared to levels in the glacial streams. The causes are still under investigation, but the cold condensation effect cannot be excluded, suggesting regional or long-range transport and followed by efficient deposition of these chemicals to the high alpine environment.

Factors deciding on environmental transport and storage of steroid hormones can be determined from computational modeling, without necessity of performing extensive empirical studies. In our project, Quantitative Structure-Property Relationships (QSPR) technique and Multimedia mass-balance Models (MM) will be applied to evaluate environmental persistence ($P_{e}$) and long range transport potential (LRTP) of these compounds. The QSPR modeling will be employed to predict the lacking phys/chem data for drugs. Physchem properties will be, then, utilized as input parameters for Multimedia mass-balance Modeling procedure. Estimated data on $P_{e}$ and LRTP will allow ranking steroid drugs according to their environmental mobility.

We started from creating and launching a comprehensive database containing all available data related to environment transport and fate of hormonal drugs. The most significant gaps in the phys/chem data were identified. For each medicine a set of molecular descriptors that characterize different aspects of molecular structure were calculated. We obtained a matrix of molecular descriptors reflecting the structural variability in the studied medicines. Data was analyzed by means of chemometrics methods, including Principal Component Analysis (PCA), Exploratory Factor Analysis (EFA) and Cluster analysis, and the medicines were divided into classes according to their structural similarity. In the same way, we grouped medicines having similar phys/chem properties of the highest meaning for environmental transport and fate processes. Finally, we put together the two results of both grouping exercises and defined relationships between both types of the similarity (structural similarity and similarity in properties). This led us to conclusions, useful for defining applicability domains for novel QSPR-MM models.

**TUPC2 - What is the current state of the science on the fate, exposure, and effects of pharmaceuticals in the environment?**

**TUPC2-1**

Towards developing QSPR-MM combined model to predict environmental fate of hormonal drugs

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2National Institute for Environmental Studies, Tsukuba, Japan

Natural and synthetic steroids are extensively used in medicine and veterinary, especially in hormonal replacement therapy, oral contraception and athletic performance enhancement. The large, and still growing consumption of these medicines throughout the world placed them among the chemicals considered by environmental scientists. Steroids have been detected in many samples of purified water introduced to natural water bodies. Their presence in drinking water, which has been also reported, pose potential risk not only for ecological system but also for human health. Therefore, a comprehensive environmental risk assessment for hormonal drugs is required.

Factors deciding on environmental transport and storage of steroid hormones can be determined from computational modeling, without necessity of performing extensive empirical studies. In our project, Quantitative Structure-Property Relationships (QSPR) technique and Multimedia mass-balance Models (MM) will be applied to evaluate environmental persistence ($P_{e}$) and long range transport potential (LRTP) of these compounds. The QSPR modeling will be employed to predicting the lacking phys/chem data for drugs. Physchem properties will be, then, utilized as input parameters for Multimedia mass-balance Modeling procedure. Estimated data on $P_{e}$ and LRTP will allow ranking steroid drugs according to their environmental mobility.

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**TUPC2-2**

Fate of 101 pharmaceuticals in sewage treatment plants, receiving surface waters and exposed biota

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Pharmaceuticals have been found in various environmental matrices worldwide in concentrations that range from low nanogram up to milligram per liter. Pharmaceuticals enter the environment primarily through our sewage systems since many pharmaceuticals are not metabolized completely but excreted unchanged. Pharmaceuticals are potentially toxic to aquatic and terrestrial organisms and therefore create an increased focus on the potentially negative effects on the environment. The purpose of the present study was to investigate the fate of selected pharmaceuticals in sewage treatment plants (STPs) as well as investigate the levels of these pharmaceuticals in receiving surface waters and exposed biota. Selection of pharmaceuticals was based on ecotoxicological criteria (criteria; primarily potency and potential to bioconcentrate, and a total of 101 pharmaceuticals were included in the study).

92 of the 101 pharmaceuticals were detected in the STP influent at least one STP, of the four Swedish STPs included in this study and 85 of the 101 pharmaceuticals were detected in the effluents. Removal efficiencies, daily loads and sludge concentrations from all four STPs are also presented.
Fifty-three pharmaceuticals were detected in the surface water samples in the range from low ng/L up to 1.8 µg/L. Measured surface water concentrations were evaluated by comparing them to critical environmental concentrations, i.e., the water concentration that is expected to cause a pharmacological effect in fish. This evaluation showed that three pharmaceuticals in these samples are expected to cause a pharmacological response in fish exposed to these waters.

Twenty pharmaceuticals were detected in the three pooled biota (perch) samples taken in the receiving surface water included in the study. Concentrations were in the low µg/Kg range and highest detected levels were found in the perch caught in close proximity to the discharge point of an STP. Detected pharmaceuticals correlate to fish surface water concentrations and previous reports on the occurrence of pharmaceuticals in biota.

TUPC-3 Evaluation of the mammalian plasma model to predict pharmaceutical responses in fish
L.A. Constantine¹, D.B. Huggett²
¹Pfizer Inc, Groton, United States of America
²University of North Texas, Denton, United States of America

Throughout the drug research and development processes, a considerable amount of data are generated to understand receptor-binding based modes of action and human pharmacokinetics including human therapeutic plasma concentrations (HTPC). Evidence of the conservation of enzyme/receptor activity across species, including mammals and the human medicinal mussel, has been shown (Jackson et al. 2003). This theoretical model is intended to facilitate prioritization of the potential impact of human pharmaceuticals on fish by comparing the HTPC to the theoretical Fish steady state Plasma Concentration (FSSPC) to determine an Effect Ratio (ER). The lower the ratio, the more likely the drug will elicit a pharmacological response in the fish. An evaluation of this model using several pharmaceuticals representing multiple therapeutic areas will be presented. To understand potential pharmacological effects, early life stage (ELS) studies were conducted using the fathead minnow. Measured fish plasma concentrations were obtained following exposure of catfish to the same exposure concentrations used in the ELS studies. These data will be presented and applied to the theoretical Mammalian Plasma Model to demonstrate the value of the model as a prioritization tool for pharmaceutical responses in fish.

TUPC-4 Metabolism and bioconcentration of the human pharmaceutical amiodarone
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¹University of North Texas, Denton, United States of America
²Pfizer Global Research and Development, Groton, United States of America

Fluoxetine, propranolol and their mixture, and fluoxetine (FX) and propranolol (PROP), alone or in combination (MIX) on Mediterranean mussels, Mytilus galloprovincialis. The aim of this study was to assess the metabolic and toxicological alterations induced by fluoxetine (FX) and propranolol (PROP), alone or in combination (MIX) on Mediterranean mussels, Mytilus galloprovincialis. The mechanisms involved were assessed by evaluating the hemocyte populations, lysosomal enzymes (lysozyme, cathepsin B) and oxidative stress (GSH, GSSG, DHE), and analyzing the effects on mussel responses, both physiological and biochemical.

TUPC-5 Influence of pH on abiotic transformation and toxic potency of pharmaceutical NSAIDs and SSRIs
M.L. Bostrom, E. Larson, J.A. Jonsson, O. Berglund
Lund University, Lund, Sweden

Influence of pH on abiotic transformation and toxic potency of pharmaceutical NSAIDs and SSRIs
M.L. Bostrom, E. Larson, J.A. Jonsson, O. Berglund
Lund University, Lund, Sweden

Environmental pH may influence bioavailability and toxicity of weak acid or base pharmaceuticals. Here we test the influence of pH on abiotic transformation, i.e. hydrolysis and photodegradation of pharmaceuticals, and toxic potency of active ingredients and transformation products of NSAIDs (weak acids) and specific serotonin reuptake inhibitors SSRIs (weak bases). Half-lives in water were determined for ibuprofen, diclofenac, naproxen, ketoprofen, sertaline and fluoxetine in a laboratory set-up at three different pHs.

The presence of pharmaceuticals in the environment has become an important topic of discussion with respect to bioconcentration and bioaccumulation of these contaminants. There has been considerable discussion on the use of mammalian data and techniques to better estimate the potential for pharmaceuticals to undergo absorption, metabolism and elimination in fish. However, robust data sets containing fish in vitro metabolism and bioconcentration data are not available for human pharmaceuticals. Using the representative cardiovascular drug amiodarone, in vitro (59 and hepatocyte) trout liver metabolism studies were conducted to evaluate the loss of amiodarone and metabolite formation over time. In addition, three flow-through fish bioconcentration studies were conducted with exposure periods ranging from 7 to 28 days.

A comparison of data sets will be presented highlighting the limitations and strengths of 59 and hepatocyte assays within the context of actual bioconcentration data.

TUPC-6 Combined effects of acidification and emerging contaminants on immune parameters of the mussel Mytilus galloprovincialis
M. Munari, V. Riedl, G. Benetello, V. Matozzo, M.G. Marin
University of Padova, Padova, Italy

Pharmaceuticals are designed to have specific (therapeutic) effects at low doses and might have side effects at high doses or after prolonged exposure, which could be induced also in non target organisms. In parallel with studies on specific effects at low doses and might have side effects at high doses or after prolonged exposure, which could be induced also in non target organisms. In parallel with studies on specific effects at low doses and might have side effects at high doses or after prolonged exposure, which could be induced also in non target organisms. In parallel with studies on specific effects at low doses and might have side effects at high doses or after prolonged exposure, which could be induced also in non target organisms. In parallel with studies on specific effects at low doses and might have side effects at high doses or after prolonged exposure, which could be induced also in non target organisms. In parallel with studies on specific effects at low doses and might have side effects at high doses or after prolonged exposure, which could be induced also in non target organisms.

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antibiotic load. Other xenobiotics are certainly also involved. The whole effluent had similar effects than the mixture of antibiotics and the filtered effluent on phagocytosis, ROS and thiol production. However it increased NO production and lysozyme activity, contrary to the filtered effluent, indicating an important role of bacteria in these responses.

TUPC3 - Occurrence, fate and impact of atmospheric pollutants on environmental and human health

TUPC3-1

Estimating the loss of life expectancy attributable to PM2.5 emissions in Europe with the use of high special resolution modelling

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References


TUPC3-2

Long-term metolachlor and Atrazine Volatilization monitoring at the catchment scale

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The emission and transport of pesticide vapor from bare soil surfaces constitutes an important pathway that is not well understood. Pre-emergent herbicides typically used for corn production include metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylpropyl) aceticamide] and atrazine [6-chloro-N-ethyl-N’-(1-methylethyl)-1,3,5-triazin-2-amine]. Loss of these compounds bounds decrease of bioluminescence. The results are expressed as the ratio of luminescence. The protocol has been standardised, the ISO standard was issued recently (ISO 21338:2010). Luminescence intensity is evaluated in a kinetic mode. The protocol applicable for testing the toxicity of solid and/or coloured samples. In parallel with the Flash system, genotoxicity was assessed using the SOS-chromotest[TRADEMARK]. This is a colourimetric test, which employs a genetically modified E. coli strain (PQ37), and uses the cells own mechanism, (the SOS repair mechanism) to detect genotoxicity.

In case of ecotoxicity analysis, the toxicity of the exhaust gas samples are given as EC50 values. EC50s are expressed as % of the initial concentration. For the tested samples, vehicle industry, energetics and environment in the Middle- and West-Transdanubian Regions of Hungary.

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References


TUPC3-3

Ecotoxicity and genotoxicity assessment of diesel engine emission


University of Pannonia, Veszprém, Hungary

Air pollution of the world’s big cities is a constant problem and source of serious health damages. The concentration of pollution is highly depends on traffic. We examined ecotoxicity and genotoxicity of twelve different diesel vehicles.

Ecotoxicity tests were carried out using the ABOATOX Flash System. This test based on measurement of bioluminescence. The test organism is Vibrio fischeri, which is a bioluminescent bacteria. As the bacterial suspension is injected to the sample, the luminous intensity increases, to a peak (maximum) within 30 s (that is why the system is called Flash). Toxicity of the sample determines the peak intensity. The results are expressed as the ratio of luminescence. The protocol has been standardised, the ISO standard was issued recently (ISO 21338:2010). Luminescence intensity is evaluated in a kinetic mode. The protocol applicable for testing the toxicity of solid and/or coloured samples. In parallel with the Flash system, genotoxicity was assessed using the SOS-chromotest[TRADEMARK]. This is a colourimetric test, which employs a genetically modified E. coli strain (PQ37), and uses the cells own mechanism, (the SOS repair mechanism) to detect genotoxicity.

In case of ecotoxicity analysis, the toxicity of the exhaust gas samples are given as EC50 values. EC50s are expressed as % of the initial concentration. For the tested samples, vehicle industry, energetics and environment in the Middle- and West-Transdanubian Regions of Hungary.

Present publication was realized with the support of the project TÁMOP-4.2.1/B-09/1/KOVN-2010-0003: Mobility and Environment: Researches in the fields of motor vehicle industry, energetics and environment in the Middle- and West-Transdanubian Regions of Hungary.

TUPC3-4

Specific toxic effects of six size-fractions of PM10 from urban locality

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TUPC3-5

Analysis of brominated and organophosphate flame retardants in indoor environment from Pakistan and Kuwait

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SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
Dust samples were collected from homes living room floors (n = 15 per country) and from cars (n = 15 per country). We aimed at comparing the two micro indoor environments for the selected regions. Dust samples were extracted by a combination of ultrasonication and vortex, followed by cleanup and fractionation by solid-phase extraction (SPE). PAHs and OPFRs were analyzed by 6890 Agilent (Palo Alto, CA, USA) gas chromatography (GC) coupled to 5973 mass spectrometer (MS) operated in electron capture negative ionization (ECNI) mode, while OPFRs were measured by GC/MS in electron impact (EI) mode. The separation and determination of α-, β-, and γ-HBCD isomers was achieved using a dual pump Agilent 1100 Series liquid chromatograph coupled to an Agilent 6410 triple quadrupole MS system operated in the electrospray negative ionization mode. Flame retardants (PBDEs, NBFRs, and OPFRs) were measured at various concentrations and with different detection frequencies in the analyzed indoor dust samples. Different exposure scenarios were calculated using the 5th, median and 95th percentile concentrations. Exposure scenarios were calculated assuming 100% absorption of intake dust, 70 kg body weight (bw) for adult and 12 kg bw for toddlers, mean dust ingestion (adults 20 mg/day; for toddlers 50 mg/day) and high dust ingestion (adults 200 mg/day; for toddlers 200 mg/day). Exposure assessment values were lower than RfD values. However, the presence of these chemicals in our indoor environments emphasizes the need to evaluate their health implications for human use.

TUPC3-6 Evaluating exposure and risk to non-smokers from tobacco smoke PAH residues on smokers T.E. Ashley*, T.J. Fleming*, C. Anderson*, L. Quast*, D.J. Velinsky*, S. Amin†
1University of Pennsylvania, Philadelphia, United States of America
Academy of Natural Sciences, Philadelphia, United States of America
Polycyclic aromatic hydrocarbons (PAHs) are a suite of potentially carcinogenic organic compounds generated during the incomplete combustion of carbon-containing material such as tobacco. Though PAHs have been widely studied in second hand smoke, their existence in third hand smoke, the residue adhering to the smoker and smoking environment after combustion. The purpose of this study was to quantify the levels of various PAHs in tobacco smoke residue, or third-hand smoke, and assess whether these concentrations are significant as a vector for exposure to nonsmokers, especially children. To quantify PAHs, levels in indoor dust, test subjects’ hands used to hold the cigarette were swiped before and after smoking with alcohol-saturated cotton gauze. PAHs were subsequently Soxhlet extracted with hexane. Hand swipes were analyzed for 20 PAHs using gas chromatography mass spectrometry (GC-MS). Smokers’ hands have significantly higher PAH levels than nonsmokers. For example, after only one cigarette smoked under ambient conditions, total PAHs were as high as 200 ng/hand for smokers compared to <40 ng/hand for nonsmokers. PAH residues on smokers’ bodies (and apparel) may represent a potential source of third-hand smoke exposure to nonsmokers, particularly children, and indoor environments.

TUPC3-7 In vitro endocrine-disrupting potential of particulate or gaseous phases of air extracts from indoor or outdoor Paris urban areas on the thyroid or estrogen receptor-mediated transcriptional activity L.O. Aïdou*, A.C. Collier*, F. Alliot*, M.B. Bimbot†, V.H. Huret*, M.C. Chevreul†, Y. Levit1
Université Paris-Sud 11 - CNRS - AgroParisTech, Château-nay-la-bry, France
EPHE - UMR Système Paris 7 6169, Paris cedex 05, France
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2Service de santé en milieu professionnel, 12 rue Jean Arthuis, 75018 Paris, France
Chronic contamination of the atmosphere by a number of endocrine-disrupting compounds (EDC) involves new societal concerns related to the importance of the constant human exposure to trace chemical mixtures and its possible subsequent effects upon health. To date, this question remains poorly documented, especially in France where only a few studies have been conducted on indoor air particularly on the comparison of indoor and outdoor contamination. Contamination of air has been evaluated primarily by chemical analyses of indicator contaminants, and mainly on compounds associated with particulates. However, gaseous phase of ambient air can also represent a potential source of EDC. In this context and as a first approach, we assessed the in vitro estrogenic and thyroid disruptive potential of both the particulate and gaseous phases fractions of indoor air extracts from Paris. Measurement: indoor air samples were collected in 20 indoor areas from the two cellular transaction assays, MELN and PC-DR-LUC respectively, which were previously used in our laboratory to characterise endocrine-disrupting activity of water sources and sediments in the greater Paris area. Four samplings were realized, and selective extractions of EDC potential were performed. Among the tested air extracts, the indoor gaseous phase was the more active in both transactivation assays, estrogenicity being quantifiable for all the samplings compared to thyroid activity. Extracts endocrine-disrupting potential was in decreasing order: indoor gaseous phase > outdoor gaseous phase > particulate phase, in a significant manner for the estrogenic effects, and as a tendency for the thyroid effects. Moreover, these differences of activity between extracts were correlated with those of cellular toxicity induced by concentrated extracts, indoor gaseous phase showing then the highest toxic content. Our results are in accordance with literature, i.e. ambient air gaseous phase induces more endocrine disruptive potential than the particulate phase. Until now, the thyroid-disrupting potential of ambient air extracts has not been characterized. This is the first report of occurrence of endocrine-disrupting activity of ambient air in France. In perspective of these preliminary results, other and more sampling campaigns could be conducted in different everyday life locations taking into account two different populations, adults and children. Effects of air extracts could also be evaluated on other endocrine endpoints.

TUPC3-8 Air quality evaluation in various indoor environments in the Lancaster University A. Katsiyannou*, H. Roberts*, A. Birgul†, A. Cincinelli†, P. Leva*, A.J. Sweetman†, K.C. Jones2
1Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom
2Lancaster University, Lancaster, United Kingdom
European Commission JRC Centre, Ispra, Italy
The present study was aimed at evaluating the air quality in a number of indoor environments in the Lancaster University, in Lancaster, United Kingdom. The study covered a wide range of sites associated to work (laboratories, office, library), recreation (sports centre, bars), private life (residences, laundry) activities. Target chemicals were volatile organic compounds (VOCs) of concern, including BTEXs (Benzene, Toluene, Ethylbenzene, Xylenes, Styrene), terpenes, siloxanes etc. The daily exposure of students/ university staff to VOCs was also estimated.

Hexane and toluene were the most abundant VOCs detected in Environmental Science laboratory, owing to the massive usage of solvents for regular extraction and analysis purposes.Limonene exhibited high concentrations in rooms and public places (cafes and bars), being consistently >100 μg/m3 reflecting the impact of the continuous cleaning routines. Hexane and toluene were the most abundant VOCs detected in Environmental Science laboratories, owing to the massive usage of solvents for regular extraction and analysis purposes. Limonene exhibited high concentrations in rooms and public places (cafes and bars), being consistently >100 μg/m3 reflecting the impact of the continuous cleaning routines. VOCs, calculated as the sum of VOCs occurring chromatographically between n-Hexane and n-hexadecane and quantified as toluene equivalents, were compared to existing risk assessment values. Significant differences were noticed with respect to existing guidelines. Indoor environments in this study have been shown to be a potential (third-hand) source of VOCs.

TUPC4-1 Community effect of activated carbon amendment to aquatic sediments D. Kowalczyk, M.I. Rakowska, J.T.C. Grotenhuis, A.A. Koelmans
Wageningen University, Wageningen, Nederland
Many in situ and ex situ studies demonstrated the high potential of activated carbon (AC) to reduce aqueous concentrations of hydrophobic organic contaminants in aquatic sediments. However, the application of AC in sediment remediation also should be ecologically safe. Accordingly, there is a need for information on possible negative effects of AC application on the ecology of aquatic ecosystems, e.g. potential impacts on benthic communities. The aim of the present study was to check colonization rates of AC-treated sediment by estimating species diversity and abundance as a function of time. No statistical difference was found in the number of taxa after 3- and 15-m colonization of AC. The number of taxa was significantly higher in AC-treated sediment compared to control sediment. Therefore, the use of AC for in situ remediation of contaminated sediments might be considered.

TUPC4-2 Sequestration of sediment associated PAHs with granulated activated carbon (GAC) I. Kowalczyk, D. Kowalczyk, T. Grotenhuis, A.A. Koelmans
Wageningen University, Wageningen, Nederland
River and marine sediments are contaminated with a wide variety of hydrophobic organic compounds (HOC), which are recalcitrant for degradation and pose a substantial risk to aqua life. Our current tools aim at the remediation and implementation of safe and cost-effective remediation technologies based on activated carbon (AC) addition. Whereas AC already has been applied to immobilize HOCs in bed sediments, the intensive removal of HOCs from sediments using granulated coarse AC (GAC) may constitute an important next step in the development of AC related remediation technologies. Therefore, we determined the capacity of GAC to reduce freely dissolved organic contaminants in sediment natively contaminated with polycyclic aromatic hydrocarbons (PAH). The obtained in situ sorption coefficients for GAC relate to binding of PAH in the presence of sediment, that is, accounting for any effect of organic matter fouling or sediment sorption competition.

TUPC4-3 Biological effects of carbon amendments in PCB contaminated sediments on Lumbricus variegatus I. Rakowska, D. Kowalczyk, T. Grotenhuis, A.A. Koelmans
Wageningen University, Wageningen, Nederland
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Carbon amendments are being studied to be used as an in situ remediation method for contaminated sediments. Most studies have focused on the efficiency of the added amendment in reducing the chemical availability of the contaminants. Few studies have been directed to follow the potential adverse effects of these amendments. The contaminated sites may have viable benthic community and therefore remediation actions can disturb the ecosystem. On the other hand, bioaccumulation in the food web results in a situation where the organisms in the higher trophic levels are at risk and/or they cannot be used for human consumption, which increases the remediation pressures. Therefore, the best methods for bioavailability and potential adverse effects of restoration measures needs to be evaluated. In this study, we assessed the effects of activated carbon and biochar in PCB contaminated sediments on feeding, growth and reproduction of an experimental organism Lumbriculus variegatus. We used coal based activated carbon and wood based biochar, which we mixed in separate experiments to the sediments. In addition, we tested if cleaning (hot water, solvent extraction) of activated carbon would affect the measured parameters. Activated carbon had sediment specific adverse effects on feeding and growth. The adverse effects of biochar were minor than those observed with comparable doses of activated carbon. The pre-treatment of the activated carbon by hot water or solvent extraction had only minor effects on the measured biological parameters. The ecological consequences inflicted by carbon amendments should be considered sediment specific.

**TUPC4-4 Bioavailability reduction with activated carbon amendment: comparison of field results and expected optimal conditions**


University of Eastern Finland, Joensuu, Finland

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theory that HOCs are predominantly bound to charcoal-like particles in natural sediments [2]. Today, these theories are confirmed by multiple studies, which found that water concentrations were reduced by 80% to 99% for PCBs, by 60% to 94% for PAHs, and by 67% to 83% for DDTs. The published data show that by reducing the flux of HOCs to the aqueous phase, sediment AC-amendment also reduces the bioavailability of these pollutants and biodynamic modeling may be used to estimate tissue concentrations. Having established that AC amendments can effectively reduce bioaccumulation, the attention has focused lately on possible secondary effects of AC on the health of benthic organisms. While AC amendment reduces the availability of HOCs, other organic matter, including dissolved organic carbon and nutrients, may become less available as well. However, to the extent that those secondary effects have been reported, they were variable across benthic species and sediments tested and results have to be interpreted carefully regarding the actual implications for field conditions. For PCB polluted sediments, bioaccumulation decreases with increasing AC dose and with smaller AC particle size. An illustrative application range for in-situ amendments of 3-4% with 70-200 µm AC resulted in a reduction of PCB bioaccumulation by approximately 80-90%. The biodynamic modeling approach predicts PCB bioaccumulation reasonably well and may help in the estimation of expected remedial success for in-situ amendments when monitoring data are not available. Currently, observed secondary effects of AC on benthic organisms are non-toxic, limited to egestion, lipid content and growth, and are not highly significant compared the benefit AC achieves by reducing exposure to persistent and bioaccumulative pollutants.

TUPC5- Are Environmental Specimen Banks ready to face future challenges of environmental chemistry and regulatory toxicology?

TUPC5-1
Derivation of dynamic reference values for the assessment of contaminant concentrations in fish of German freshwater ecosystems using data of the German ESB
D. Teubner, P. Müller, M. Paulus
Universität Trier, Trier, Germany

Accumulation indicators are widely used in biomonitoring studies and programs, respectively. The contaminant concentrations in biological matrices of different sampling sites can show regional differences and time series can show temporal changes. However, in terms of relevance to biological systems, assessing contaminant concentrations is very difficult. Asessment by sublethal effect concentrations or by environmental quality standards is only possible in exceptional cases.

TUPC5-2
Retrospective analysis of perfluorinated alkyl acids in marine mammals from Alaska
NIST, Gaithersburg, United States of America

Temporal trends examined in both species indicated that most PFAA concentrations increased in belugas from 1986 to 2006 and in northern fur seal livers were collected from one location, St. Paul Island. Long-chain PFCAs, (9 to 14 carbons), and PFOS were detected in most of the samples-

TUPC5-3
Environmental specimen banking in China: necessity and development
X.Z. Meng, Y.L. Qiu, Q.H. Huang, Y. Liu, D.Q. Yin, J.F. Zhao
Tongji University, Shanghai, China

Environmental specimen banking in China has traditionally dispersed in several different programs each with its own sample storage strategies and data processing systems. In spite of numerous useful series of samples and data, researchers may often be unaware of the existence of relevant material or specimens obtained. To fulfill these needs the Finnish Forest Research Institute (SYKE) and the Ministry of Education established a research and development project “Environmental specimen banking and co-operation between different institutes in Finland”.

TUPC5-4
Environmental specimen banking development in Finland
E.J. Kuhn, J.P. Poikolainen, S.K. Kemppainen
Finnish Forest Research Institute, Muhos, Finland

1. Introduction
Environmental sampling in Finland has traditionally dispersed in several different programs each with its own sample storage strategies and data processing systems. In spite of numerous useful series of samples and data, researchers may often be unaware of the existence of relevant material or specimens obtained. To fulfill these needs the Finnish Forest Research Institute (SYKE) and the Ministry of Education established a establishment of environmental specimen banking and co-operation between different institutes in Finland”. The project aim was to develop the storage and use of existing specimen material in scientific research and education.

2. Specimen banks in Finland
In Finland there are at the present moment two environmental specimen banks, Paljakka and Hakaninmaa, established by Finnish Forest Research Institute (Metla) and Finnish Environment Institute (SYKE). SYKE coordinates and participates in monitoring of harmful substances in aquatic and terrestrial environments. Metla established a network of 3009 permanent monitoring sites all over the country in 1985-1986 for forest inventory and monitoring environmental changes. During the first two years 6 877 moss, lichen and pine bark samples were collected. The Paljakka ESB was store to increasing amount of samples in 1994. Stored samples originated from several national and international projects like UNECE/ ICP Vegetation and ICP Forests. At this moment the amount of samples is estimated to exceed 700 000.

3. Development needs
There is a clear need to develop information availability and co-operation in the utilization of existing environmental data and material. This was also expressed at the International Environmental Specimen Banking and Information Management Seminar. Several topics were emphasized for further discussion:

- Data sharing should be developed and updated continuously.
- Existing sample material is not intensively utilized.
- When environmental monitoring is being harmonized, end-users should be taken account.

TUPC5-5
Long-term monitoring by trees within the German ESB
G.E. Wagner1, C. Weimann1, D. Teubner1, M. Bartel-Steinbach1, R. Klein1, K. Tarricone1, T. Weismann1, A. Koerner1, H. Ruedel1, M. Paulus1
1University Trier, Trier, Germany

Biometrical and analytical characterization of systematically sampled specimens for the German Environmental Specimen Bank (ESB) can be used as a real-time monitoring system for the verification of the long-term comparability of the applied sampling system. Samples from trees representing environmental contamination on the ecological level of primary production are sampled within 9 representative areas of different exposure in Germany for up to 24 years. Samples were taken each year, in some cases every second year applying standardized sampling procedures.

A thorough analysis of the data comes to the following results:
- Increased needle coverage over 25 years demonstrate improved vitality of spruce trees near the urban-industrial conurbation of Saarbruecken
- Many of the analyzed substances (PAHs, Pb, As) in conifer shoots from forests near urban-industrial conurbations declined by almost a factor 10 or more from 1990 to
2000.
- Sulfur in the same samples decreased to about 50% of their former concentrations near Leipzig and about 80% near Saarbruecken.
- PCB concentrations decreased to about 20% from 1985 to 1997 near Saarbruecken and to about 50% from 1991 to 2000 near Leipzig.
- From 1990 to 2010 the concentrations of PAHs, Pb, As, S and other substances in conifer Shoots from forests near urban-industrial contaminations continued to decrease, but much slower.
- In remote regions like the National Parks (NP) of Bavarian Forest and Berchtesgaden, PAHs and Pb did not decrease any more after 2000.
- In samples from the NP Bavarian Forest concentrations of PAHs, Pb, Ti, and As were 1.5 to 5 times higher than in samples from the NP Berchtesgaden.
- The NP Bavarian Forest is a remote area, but, in opposition to the NP Berchtesgaden, it is exposed to long-range transport of air pollutants from southern Germany and the Czech Republic.
- Since 2000 concentrations of high molecular PAHs, Pb and As in samples of the NP Bavarian Forest tend to exceed more and more those of samples from forests near urban-industrial agglomerations of Saarbruecken and Leipzig.

TUPC5-6
The potential for a UK environmental specimen bank
Centre for Ecology and Hydrology, Lancaster, United Kingdom

- Manchester has invested approximately £16 million per year. However, there is no current UK-wide strategic coordination of this investment. Although the United Kingdom Environmental Observation Framework (UK-EOF) catalogues environmental observations made for and by the UK, it does not currently catalogue specimen collection. This poster describes a short study to: (i) gather information on current specimen collection and archiving in the UK and (ii) investigate the potential for a UK Environmental Specimen Bank (UK-ESB) that would provide links and strategic coordination between different collections.

TUPC5-7
Perfluorinated compounds in liver from wild otters (Lutra lutra) from Sweden and Norway, collected between 1972 and 2011 - a new threat to the otter population?
Institute of Environmental Research, Stockholm, Sweden

- Swedish otters had similar or much higher concentrations of PFOS than what was reported from the river otter (Lutra canadensis) in North America (25-994 ng/g liver w.w.) and the American mink (Mustela vison, up to 5140 ng/g w.w.). The highest concentrations were found in otters from the south-central more urban parts of Sweden.
- No obvious adverse health effects have been studied within this study. All otters were frozen before sent to the museum or NIVA, so histopathology was not possible to be done.

TUPC5-8
The US Pacific Islands Region biorepository and environmental specimen bank
J. Moors1, J. Pugh, R. Becker, E. Bryan, S. Vinder Pol, M. Keller, R. Kucklick, D. Day, A. Wise
National Institute of Standards and Technology, Charleston, United States of America

- The NP Bavarian Forest is a remote area, but, in opposition to the NP Berchtesgaden, it is exposed to long-range transport of air pollutants from southern Germany and the Czech Republic.
- From 2000 to 2010 the concentrations of PAHs, Pb, Tl, and As are 1.5 to 5 times higher than in samples from the NP Berchtesgaden.
- PCB concentrations decreased to about 20% from 1985 to 1997 near Saarbruecken and to about 50% from 1991 to 2000 near Leipzig.

TUPC6-1
In silico to the chemical ecology of Myriophyllum spicatum adapted for ecotoxicological assays
E.M. Gross
Université de Metz, Metz, France

- Myriophyllum spicatum, a facultative submerged aquatic plant, is frequently used in bioassays to test new dicotoryl submerged species in aquatic systems, flowing and stagnant waters as well as brackish systems. Since 1991, we have developed axenic cultures of M. spicatum and other milfoil species in our laboratory. Now, Myriophyllum has been proposed as future OECD aquatic bioassay system in ecotoxicology.
- In the past we investigated the allelochemical role of hydrolyzable polyphenols, which are present in this species in exceptionally high concentrations. Among them are also so called ellagitannins, well known for their possible antioxidant or pro-oxidant activity. Milfoli polyphenols act as natural herbicides and pesticides against competing algae and aquatic insect larvae. The efficacy and specificity of these biocidal metabolites is specifically affected by abiotic and biotic stresses. Different environmental conditions such as light and nutrient availability or the presence of bacteria affect not only the concentration and but also the composition of polyphenols in M. spicatum.

TUPC6-2
Modified sediment-free Myriophyllum biotest
G.G. Gomori, K. Weber
European AgroSciences Ecotox GmbH, Niemern-ischelbronn, Germany

- myriophyllum aquatics is used in an unterrile water-sediment or axenic sediment-free system were performed. Here we present a test design without sediment under unsterile conditions. The pros and cons of this test design will be discussed in comparison to the water-sediment and axenic sediment-free tests.

TUPC6-3
Is toxicological sensitivity of Myriophyllum aquatics compared to Lemma minor an argument to refine risk assessment of chemicals?
T.T. Tunić1, V.Z. Knezević1, T. Marjan1, M.S. Kezunović, I.S. Teodorović1, 2
Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

- Myriophyllum aquatics is a candidate for an additional macrophyte species in refined risk assessment of chemicals. To contribute to filling in the largely missing data on relative sensitivity of M. aquatics to toxic compounds (compared to Lemma sp.), four substances were tested using both species in parallel: atrazine, isoproturon, trifluralin and 3,5 DCP. Lemma minor tests were conducted following the standard OECD protocol, while M. aquaticum was tested in water-sediment system according to draft protocol suggested for future OECD standard. Growth inhibition in Lemma tests was estimated using relative growth rates (RGR) and yield based on total number of fronds, total frond area and total fresh weight biomass. Growth inhibition of M. aquaticum was estimated using RGR based on length of the plants above sediment, total length of the plants, number of side shoots, total dry and fresh weight biomass. The only common end point for both tests is the RGR based of total fresh weight biomass at the end of the
Based on the test protocol “Guidelines for assessing the effects of pesticides on the growth of Glyceria maxima, University of Bristol, Long Ashton, Bristol, 23.05.2001” the main factor which reduced the variance was to start the test with plants having two shoots instead of plants with a different number of shoots. The control plants nearly doubled within the test period. The test substance was applied via a water phase without any renewal of the test media. Assessments of shoot length were made weekly and shoot wet and dry weight was measured at the beginning and end of the test. The results showed that this test method is appropriate to assess the effects of an herbicide on Glyceria maxima although some improvements still have to be considered (e.g. how to inhibit the algae growth in the test vessels completely). The calculated endpoints (growth rate and yield of each parameter) showed acceptable coefficients of variance, which were within 20 to 30% for the total shoot length, within 10 to 20% for wet and dry weight of the control plants after 28 days of exposure. The mean shoot length of the control plants nearly doubled within the test period. The main factor which reduced the variance was to start the test with two shoots instead of plants with a different number of shoots.

Aquatic macrophytes can decrease the availability of contaminants in mesocosms and experimental ditches. However, the experiments showed that macrophytes also accumulate metals and act as a temporary sink. Mesocosm and laboratory experiments revealed that Myriophyllum spicatum and Lemna sp. increased the DOC (Dissolved Organic Carbon) levels in the water phase and contributed to the amount of hydrophobic DOC in the water phase. The submerged macrophytes were important for binding metals and hydrophobic pollutants. Increased levels of DOC in the water phase result in lower free Cu and Zn concentrations thereby lowering the bioavailability of these metals. In conclusion, macrophytes are an important sink for micropollutants like adsorbing pesticides and metals. Besides direct effects, macrophytes indirectly influence water concentrations by generating DOC, which can bind micropollutants, and by changing the metal speciation.

Assessing the potential of Miscanthus x giganteus for fly ash revegetation and soil restoration


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Fly ash (FA) is the primary byproduct of coal-fired power stations with an annual global production that averages approximately 550 Mega tons. Due to its weight and lack of mobility, re-aggregation may spread to long distances and cause serious environmental problems. In order to stabilize this material and reduce detrimental effects, we focused our work on the establishment of a direct vegetation on ash. The objective of this study was to investigate the potential of Miscanthus x giganteus, a sterile rhizomatous grass, for fly ash revegetation and soil restoration. To evaluate its growth capacity on FA, plant development during pot experiments was compared to that of myriophyllum spicatum and Lemna sp. in the same environmental conditions. The P. A. N. content of the leachate from the bioabsorbent increased significantly. Finally, in the third stage, the Pb leached and concentrated in the leachate it was recovered by electro-deposit, achieving a maximum recovery efficiency of 93%.

Rhizofiltration, leaching and electro-recovering: an integrated approach to treat wastewater polluted with lead using water hyacinth (Eichhornia crassipes) as bioabsorbent

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This in vitro study was conducted in an integrated approach which involves biological, chemical and electrochemical processes in order to treat waters polluted with lead using water hyacinth as bioabsorbent. The research was carried out in three stages: i) bio-absorption, ii) leaching, and iii) electro-recovering. In the first stage it was determined that water hyacinth can absorb Pb bio-available forms of Pb in Pb-contaminated media and that the Pb bio-available in high concentrations (77% of total Pb) was in the organic fraction. To do this, we performed a factorial experiment crossing five densities of the common submersed macrophyte, Elodea canadensis, with five malathion concentrations in 1-L outdoor microcosms. We determined the L.E.D. (λ) values for D. magna in each macrophyte treatment using Probit analyses and tested for significant differences by comparing the overlap of the confidence intervals. Overall, we found that as E. canadensis density increased, the toxicity of malathion to D. magna declined substantially. For example, the malathion concentration required to kill 50% of D. magna was 23 times greater in microcosms containing high, but environmentally relevant, E. canadensis densities than in macrophyte-free microcosms. Additionally, we found that the rate at which microcosm water was detoxified increased with increasing malathion density. This study is the first to experimentally examine the extent to which macrophytes may mitigate the biological effects of insecticides. The results provide further support that ecological interactions can dramatically influence the effects of contaminants.
WE 001 Development of an AQUATOX ecosystem model for assessing effects of an herbicide on aquatic food webs

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The use of toxicity experiments in laboratory, mesocosm, and field applications plays an important role in pesticide risk assessment but cannot address all issues related to indirect effects mediated through species interactions within food webs. For example, as part of an application for inclusion of HerbicideX (hypothetical name) in Annex I (Article 11), available laboratory toxicity data and acute no-effect results indicated the potential for short-term effects on phytoplankton and periphyton species, but the nature and time-frame of potential indirect effects on aquatic animals, including fish, were unknown. Aquatic ecosystem modeling can be used to evaluate not only direct toxic effects of pesticides but also indirect effects mediated through trophic interactions of species (e.g. competition and predation).

We have developed an aquatic ecosystem model using AQUATOX (version 3.0) to evaluate the potential direct and indirect ecological effects of HerbicideX on freshwater systems. We report here on the design and calibration of AQUATOX to represent mesocosms originally intended for assessing community-level effects of HerbicideX. Physical characteristics, nutrient loading, and major biotic groups in the AQUATOX mesocosm-mimic model corresponded to those of the HerbicideX-study mesocosms. Biological communities included phytoplankton, periphyton, zooplankton, benthic and epiphytic macroinvertebrates, and macrophytes, but not fish as the latter were absent from the mesocosms. To match characteristics of the mesocosms, our mesocosm-mimic model is spatially aggregated (i.e., assumes spatial homogeneity in the water column) and lacks immigration and emigration. The model was parameterized using data from the HerbicideX-study mesocosms, from the AQUATOX internal library, and from the open literature. Information on toxicity and environmental fate of HerbicideX was obtained mainly from the Draft Assessment Report developed by the responsible Rapporteur Member State. Overall, the model is well calibrated (generally within a factor of 2-3) to the mesocosm data, particularly for HerbicideX concentration and biological variables.

We conclude that aquatic ecosystem modeling complements empirical approaches to risk assessment by allowing efficient evaluation of indirect effects, testing of multiple exposure scenarios, and assessment of potential long-term effects and recovery times.

WE 002 Prediction of ecological impact of eutrophication and pesticides in a Luxembourgish stream using AQUATOX: linking eco-toxicological models and biological indices

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One of the main current challenges in the implementation of the WFD is the identification of the pressures that cause the deterioration of ecological status of water bodies and the capability to predict the current ecosystem evolution upon mitigating these pressures. Different modelling approaches, linked to monitoring strategies, have been recently proposed to support stakeholders, but these models are often not integrative and not predictive and therefore do not provide direct, indirect and synergistic effects of nutrients and pesticide have been analysed to make meaningful prediction with AQUATOX, we related nutrients and pesticides loads to specific community structures, reflecting the ecological state. Simplified food webs corresponding to lead species of the saprobic index classification and matching the corresponding water quality classes have been built within AQUATOX, calibrating nutrients and contaminant tolerance. Based on the expected macroinvertebrate assemblies, the simulations in AQUATOX estimate the presence of indicator species for the different saprobic classes, under the corresponding pollution level.

This approach allows to predict ecological status of water bodies in terms of water quality and to distinguish between nutrients/organic and toxics effects as well as between direct and indirect effects.

WE 003 Species Sensitivity Distribution (SSD): Different data validation approaches and their influence on the final SSD curves for selected herbicides

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Species Sensitivity Distribution (SSD) is becoming a common method for evaluation of ecological risks of chemicals. However, there is still no clear agreement about the standard procedure of SSD curve derivation. Many different statistical and data validation approaches have been suggested. In the present work we study different commonly used data validation approaches, and their influences on the final SSD curves, HC5 and PAF / msPAF values. The study has been prepared using data on seven herbicides relevant for the aquatic environment. Ecosystem data were derived from databases (e.g. USEPA ECOTox) and literature. We have compared acute vs. chronic toxicity data, saltwater vs. freshwater toxicity data, using all endpoints vs. selection of the most relevant endpoints, and finally using data for different taxons. We also compare two approaches of using database records: 'using all data' and 'detailed validation and searching for duplicates and errors'. These comparisons have been applied to monitoring data from the Scheldt River basin and data from herbicide applications in Belgium, where PAF and mPAF values have been calculated. We discuss statistical evaluation of the differences, as well as influence of different steps in the data validation on the overall outcomes of SSD modelling. [Supported by the EU FP7 project AQUAREHAB and by the project CETOCOEN (no. CZ.1.05/2.1.00/01.0001) from the European Regional Development Fund.]

WE 004 Fuzzy approach for risk assessment of brominated flame retardants in aquatic ecosystems


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Brominated flame retardants (BFR) are pollutants that represent a threat to both human health and environment due to their industrial use, their persistence and their ability to bioaccumulate and bioconcentrate in food chains, especially in the aquatic one. For the last ten years contamination levels for this type of compounds have been reported for European, North American and Asian human tissue, sediments and biota samples [1-3]. However, monitoring efforts into the assessment of BFRs contamination levels in Latin America is scarce.

In this work we present a model for the evaluation of the environmental risk of BFRs in the aquatic ecosystems has been developed. It has been based on a technical application of the Fuzzy Theory [4]. In particular, three interconnected Fuzzy Inference Systems (FIS) have been created through the use of the Fuzzy Toolbox in Matlab. In order to improve and make the model scientifically robust, several international experts have been questioned about different information required to build the fuzzy system. Information from 38 questionnaires have been collected and statistically treated.

The model has been tested in two case studies: Ebro River basin (Spain) and Latin America (Chile and Colombia). The data gathered for each one of the two case studies correspond to different two international projects. The first one is the EU funded AQUATERRA project (2004-2009): Integrative modelling of the river-sediment-soil-groups on the Ebro River for this study data from four samples campaigns in two Ebro tributaries in north east of the Spain, the Cinca River (2002 and 2004) [5] and the Vero River (2004 and 2005) [6].

The second project is the BROMACUA funded by the BRVA Foundation. Contamination levels, bioaccumulation and biomagnification in the aquatic trophic chain are being evaluated in two ecosystems, one from Chile (San Vicente bay and Lenga estuary) and another one from Colombia (delta-estuary of the Magdalena river). Contaminant data obtained in the sampled campaigns of both projects have been used directly as input for the model. In this paper, the characteristics of the developed methodology as well as the main results of the model are presented. The main conclusions is that the sites in Spain present higher risk that the ones analyzed in Latin America.

WE 005 A simulation model of mortality, detection and sampling for anticipating performance of field surveys of plant protection products acute effects on wildlife

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SAGIR is a French national network of environment workers collecting dead or moribund animals to assess the main effects of wildlife mortality. SAGIR's surveys are based on opportunistic approaches:
- a national events survey based on opportunistic collection;
- a focus events survey based on opportunistic collection with a particular focus to environmental time and geographic scales;
- an active survey based on more intensive sampling.

Toxics examined by SAGIR concerned also Plant Protection Products (PPP) acute effects on wildlife. Although these surveys are used and advised for post-homologation studies, fatalities detected during field surveys are still difficult to relate to actual mortality because of numerous ecological and human factors leading to variation of the determined probability of site visitability.

We built an integrated model to simulate spatial dispersal, persistence time, detection and sampling of carcasses on an area. This model is used to simulate different strategies to estimate the proportion of carcasses contaminated with the PPP and/or its residues in the population of carcasses. The model is composed of three different components. In the state model, individuals of a generic species are randomly distributed on an area composed of PPP-treated and non-treated plots. Individuals are submitted to mortality rates (natural and related to PPP poisoning).

If an animal dies, its carcass has a daily probability to disappear from the area, estimated from field experimental assays. The
output of this state model is therefore a number of carcasses per plot and day.

Next, an observation model is considered. Observers have a certain probability to detect a carcass, estimated from distance sampling experiments. Finally, a sampling model with SAGIR methodologies simulations is used to assess the proportion of the actual number of carcasses, collected during an event survey or an active survey.

This kind of integrated model can be used to simulate different field search strategies and to assess material and human resources necessary to obtain a reliable estimate of PPP or residues prevalence in a population of carcasses. The difficulty to perform such field study is therefore highlighted.

WE 006

DEB model to interpret the combined effects of toxicants and environmental factors: case study on Folsomia candida
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Boron is a naturally occurring trace element that is essential to a variety of organisms. This poster will describe the available data considered in developing a freshwater PNEC for boron according to the REACH framework. Data screening based on reliability and relevancy criteria (Klimisch score) was used to select the high quality chronic toxicity data of boron according to the REACH framework. Data screening based on reliability and relevancy criteria (Klimisch score) was used to select the high quality chronic toxicity data of boron according to the REACH framework. Data screening based on reliability and relevancy criteria (Klimisch score) was used to select the high quality chronic toxicity data of boron according to the REACH framework. Data screening based on reliability and relevancy criteria (Klimisch score) was used to select the high quality chronic toxicity data of boron according to the REACH framework.

We used an existing software tool, DEB-BM, to develop an individual-based population model, based on DEB theory, of the collembola Folsomia candida, a standard species for risk assessment of soil invertebrates. The model considers the influence of temporal and spatial variation of temperature on Folsomia candida populations exposed to cadmium and also includes density-dependent effects. First results are encouraging since they qualitatively reproduce population-level data. More quantitative analyses are required. Therefore we can conclude that DEB theory, combined with individual-based population models, is a generic and powerful tool for ecological risk assessment of chemicals.

WE 007

Population-level toxicant sensitivity: stage-specific density dependence and demographic stochasticity in Cu-exposed four-beetles (Tribolium)
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Population models are needed for predicted predicting toxicants effects on population-level endpoints such as long-term abundance and extinction risk from measured individual-level endpoints such as survival and fecundity. Demographic processes such as density dependence and demographic stochasticity (uncertainty in vital rates due to random differences among individuals) can complicate predictions of population-level effects. Stage-structured populations are particularly complicated; the different life-history stages may show different sensitivity to toxicant exposure, but the most sensitive stage is not necessarily the most important endpoint for the overall population-level effect. In this study we have investigated the role of stage-specific toxicants and stochasticity in populations of the red flour beetle (Tribolium castaneum) exposed to a toxicant using a population model (T. castaneum) which was developed for T. castaneum and has been applied extensively in population ecology for two decades. Density-dependent cannibalism across stages is driving the characteristic population dynamics in this model. Demographic rates (development time, juvenile survival and reproductive rate) in Cu-exposed and control populations of T. castaneum were estimated from a multi-dimensional sampling. Each treatment included 20 replicated populations, from which we could obtain estimates on uncertainty in demographic rates. The population model was used to simulate population dynamics over multiple generations, including demographic stochasticity in reproduction and survival. The aim of this modelling study was to analyze: (1) effects of Cu-exposure on population-level endpoints such as long-term abundance and risk of (quasi-)extinction; (2) sensitivity of the population-level endpoints to the Cu-levels; (3) the role of demographic uncertainty on the population-level toxicant effects and (4) the role of demographic uncertainty for the population-level toxicant effects. Analysis of demographic rates from the simulated population time series show that density dependence dampened the long-term Cu effects e.g. on reproductive rates. Nevertheless, sensitivity analysis indicates that Cu effects on reproduction were more important for the overall population responses than changes in larval or adult mortality.

WE 008

How population models can make better use of existing toxicity test data in environmental risk assessment: a case study with musk xylene
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We use musk xylene as a case study to analyze the extent to which typical ecotoxicity test data sets produce data that can be used in population models to produce value-relevant outputs. Our analyses show that population models can be used to integrate and interpret data for different organism-level endpoints that are typically measured in standard toxicity tests. Thus, standard toxicity tests can, in principle, provide information that can feed into value-relevant assessments of chemical impacts. How toxicity data are toxic is context-specific in this case, several changes need to be made in the way that tests are prioritized and in the way that the data are reported. In addition, there needs to be agreement on the criteria that population models have to fulfill in order for them to be acceptable as a basis for risk assessment and management.

WE 009

Energetics affects toxico-kinetics: a case study of uranium toxicity on the metabolism of zebrafish, Danio rerio
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Uranium is a heavy metal that is essential to organisms. This poster will describe the available data considered in developing a freshwater PNEC for the mode of action of uranium. Care must be taken however in the interpretation of the results and especially when extrapolating to field conditions where food is sometimes limiting. Analysis of data sets becomes straightforward in this context and results are no longer conflicting. Laboratory experiments under controlled conditions give mechanistic insight into the mode of action of uranium. This poster will describe the available data considered in developing a freshwater PNEC for the mode of action of uranium. Care must be taken however in the interpretation of the results and especially when extrapolating to field conditions where food is sometimes limiting. Analysis of data sets becomes straightforward in this context and results are no longer conflicting. Laboratory experiments under controlled conditions give mechanistic insight into the mode of action of uranium.

This kind of integrated model can be used to simulate different field search strategies and to assess material and human resources necessary to obtain a reliable estimate of PPP or residues prevalence in a population of carcasses. The difficulty to perform such field study is therefore highlighted.

WE 010

Physical, biological and chemical methods for classification of arsenic and manganese bearing wastes and soils in Western Georgia and health-risk assessment
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High Ca concentrations ameliorate Zn toxicity (Santore et al., 2002) - Water chemistry was collected at locations around Ross Lake in October 2010 and July 2011 - Free ion Zn and Cu was measured using Diffusive Gradient in Thin Film (DGT) membranes - Speciation calculations were performed using WHAM and measured water chemistry - LC50 was estimated using site specific data in the Biotic Ligand Model (BLM) - Standard leaching tests (EN 12457-2) asked by European authorities to guide landfilling were first performed on ten samples of soil taken from a site recycling lead-acid batteries. This experiment determines the fraction potentially mobilized and therefore poses a risk if transferred. Physico-chemical parameters from all samples were also determined (pH, total concentrations, etc). Toxicity of leachate was estimated by Daphnia magna (OECD 202) and Alivibrio fischeri (Vibrio fischeri, ISO 11348) bioassays. Besides, four modified Escherichia coli stains with luminescence modulated by heavy metals were also used to assess the toxicity of these pollutants. Results showed that although total lead concentrations can be very high (up to 40 mg/kg), only a small fraction was solubilized. Chemical analysis and ecotoxicological bioassays were well correlated. This study highlighted the use of modified bacteria strains sensitive to metals will be useful for the end-users for environmental monitoring of contaminated sites.

Prospective assessment around a lead-batteries recycling company using ecotoxicity tests WE 013

Risks assessment around a lead-batteries recycling company using ecotoxicity testing Y. Foucault, J. C. Capowiez, J. M. Durand, E. J. Schreck, T. Rack, T. Leveque, P. Pradere, C. Dumat* STIR, CNRS, Toulouse, France
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What's causing toxicity at a zinc-copper mine site? WE 014

Does phosphorus limitation increase the toxic effect of silver on aquatic fungi and leaf litter decomposition? J. A. Arce Funck
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2Forested headwater stream functioning is intimately linked to leaf litter decomposition ensured by microbial decomposers, mainly aquatic hyphomycetes, which change allochthonous carbon to higher trophic levels. Evaluation of this process is increasingly used as an indicator of the state of ecosystems, both in terrestrial and in aquatic conditions. Yet, impacts of contaminants on this process, as well as effects of environmental conditions on the course of this process to contaminants, remain scarce. Interactions between the toxic effects of metals and phosphorus (P) level, limiting element which is one of the main drivers of detritus decomposition, have never been tested. We carried out a microcosm study with a consortium of eleven hyphomycete species to verify if 1) the decomposition process under metallic stress (presence of silver, Ag) could be affected by phosphorus level, and 2) if the Ag effect could be modified when organisms benefit from high phosphorus availability. 2), these effects being mediated by changes in aquatic hyphomycete community structure. Fifteen combinations of metal and nutrient conditions, i.e. 5 concentrations of silver nitrate (0, 0.1, 0.5, 1, and 10, and 100 µg Ag-L-1) and 3 concentrations of phosphorus (20, 100 and 1000 µg P-L-1) were tested, each treatment being replicated 4 times. Litter decomposers and fungal biomass were increased by P level. Toxic effect of Ag on decomposition was significant only at the highest concentration independently of P level. On the contrary, P and Ag level had a significant interaction on fungal biomass. Analyses of fungal community structure via PCR-DGGE showed that both P level and Ag concentrations shaped microbial communities, without significantly altering species richness. This study shows that toxic effects of Ag contamination on fungal community structure are more pronounced under P-limitation. This limitation is often encountered in headwater streams ecosystems that could make them more sensitive to metal stress. Despite alterations of community structure, P-limitation remained the most important driver of leaf litter decomposition, excepted under very high Ag concentrations. Using leaf litter decomposition as a functional indicator of state of ecosystems seems thus more sensitive to nutrient availability than to metal contamination, excepted at very high metal concentrations.

What's causing toxicity at a zinc-copper mine site? WE 015

What's causing toxicity at a zinc - copper mine site? S. A. I. M. Lee, M. L. Diamond, C. Gueguen, N. Gandhi
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Introduction

Hudson Bay Mining and Smelting (HBMS) operates a mine in Flin Flon, Manitoba CA. The mine produces Zn and Cu concentrate. Zn and Cu rich effluent from the tailings pond discharges into Ross Lake, which has been found to have extremely low populations of biota. pH in the lake averages 6.5 with occurrences of low pH. At low pH free ion Zn is more soluble and hence bioavailable. With high Zn and Cu concentrations, and low pH, it is unclear what is causing toxicity in Ross Lake.

Goals
- Determine source(s) of toxicity in Ross Lake
- Measure and model bioavailable fractions of Zn and Cu

MODELING
- Speciation calculations were performed using WHAM and measured water chemistry
- LC50 was estimated using site specific data in the Biotic Ligand Model (BLM)

FIELD SAMPLING
- Free ion Zn and Cu was measured using Diffusive Gradient in Thin Film (DGT) membranes
- Water chemistry was collected at locations around Ross Lake in October 2010 and July 2011

3. Results and discussion
- WHAM estimates of free ion metal show good agreement with Zn and Cu concentrations measured with DGTs
- Zn2+ concentrations are consistently lower than LC50 calculated by BLM, Zn not a source of acute toxicity
- High Ca concentrations ameliorate Zn toxicity (Santore et al., 2002)
- Cu2+ concentrations are consistently higher than LC50 calculated by BLM, Cu a source of acute toxicity
- pH not likely influencing Zn or Cu toxicity

Conclusions
- Cu2+ consistently causing toxicity
- Low pH likely to also stress organisms, but not due to increasing Zn solubility

Recommendations
- Explore the effect of metal mixtures on toxicity using this data set and newly available models. Determine if zinc and copper are having a synergistic effect on Ross Lake.

Acknowledgements and References


The effects of pH on acute and chronic toxicity of Pb2+ on Daphnia magna and Ceriodaphnia dubia and Biotic Ligand Model (BLM) development C. Nys, C.R. Janssen, K.A.C. De Schamphelaere

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We investigated the individual effects of pH on the chronic toxicity of Pb\(^{2+}\) on Daphnia magna and Ceriodaphnia dubia. Until now the effects of pH on the acute and chronic toxicity tests are not clear. We studied the development of a chronic test for this group of organisms. The effects of pH were determined during 48 hours acute tests and a 21 and 7 days chronic reproduction test for D. magna and C. dubia respectively. The pH experiments were conducted with 4 pH levels (6.4, 7.0, 7.6 and 8.2). Results of the acute tests for both species showed that there was limited effect of pH when considering the dissolved lead fraction. When corrected for species differences with an increasing toxic effect the free ion (Pb\(^{2+}\)) at higher pH Preliminary results of the chronic test for C. dubia suggest that there were no effects of pH on the toxicity of lead. Initial results of the chronic test with D. magna showed an increasing toxic effect with decreasing pH. Finally the results on the we are developing a BLM for the Daphnia genus.

**WE 017**

**Impact of incorporation of bioavailability on risk assessment of metals in Japanese surface waters**

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Metal speciation is a function of water chemistry including temperature, pH, organic content, and the compositions and concentrations of ions and solid phases in solution. To address the predictive methods of correctly managing the risk posed by metals to aquatic life is important to determine the effects of water chemistry on the bioavailability of metals in surface water. For this reason, we have employed the technique called diffusive gradients in thin-films (DGT) in the study of several Japanese rivers, including meta-contaminated rivers adjacent to abandoned mines, to gain information on the bioavailability of metals in Japanese water systems. The measurements of labile-metal as determined by the DGT technique suggest that the biologically available fractions of metals in urban rivers are relatively low compared with those in rivers adjacent to abandoned mines. The fractions of Zn and Cd appear to be higher than that of Cu. An inverse relationship was found between labile-Cu fraction and dissolved organic carbon (DOC). Our result suggested that large amount of Zn and Cd occurred as hydrated ions or small inorganic complexes in water of the rivers studied, whereas most of the Cu occurred as Cu-organic complexes. Moreover, we have examined the impact of incorporation of bioavailability on risk assessment of metals in various Japanese surface water types and to identify what types of Japanese surface waters and metals required to consider bioavailability when assessing risks to aquatic life.

**WE 018**

**Delivering a practical methodology to account for metal (bio)availability in the water framework directive - case studies**

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The use of the water framework directive for the aquatic environment, FWD, requires that the full test for acute (7 d) and chronic (28 d) classifications. Lead metal powder showed the solubility (> 3000 µg/l) that was above the EQS for lead. The two lead compounds are discussed. In general, the assays were performed in mass or equivalent surface loadings of 1, 10, and 100 mg/L at pH 6, 7, and 8, as appropriate, for screening (24 h) and/or longer exposure times to investigate the rate and extent to which these compounds can produce soluble ionic and other metal bearing species in aqueous media according to OECD Series No. 209 (2001). A test system that meets the specifications of this OECD protocol has been successfully implemented to examine the transformation/dissolution behaviour of a range of metal substances, including redox-sensitive metals. The aqueous test media are based on reconstituted water prepared according to ISO 6341 with pH values in the range of 5.5 – 8.5. The OECD protocol requires exact and comprehensible procedures (e.g. accurate loadings, temperature monitorability, performance of mass balance at test end, and tests were performed in accordance with GLP requirements. In addition to a strict execution of the experimental test procedure, methods for the quantification of dissolved metal ions and species were developed and applied. Element-specific determinations by ICP-OES or ICP-MS were used and validated by appropriate QA/QC measures, including the analysis of certified reference materials (CRM) and blanks. When different methods were tested, a dedicated coupling methods, i.e. liquid chromatography coupled to ICP-MS, were developed and successfully applied to separate metal species in different oxidation states. Due to redox equilibrium processes, the stabilization of the formed redox-species immediately after sampling was critical. Differences in transformation/dissolution were observed in response to varying loadings, solution pHs, exposure times and particle surface areas. Our results found a test system to be suitable with sufficient replication as well as the benefits of testing samples with narrow particle size distributions. The relevance of these findings for read-across and the respective classification and labelling will be discussed as well as strengths and weaknesses of the current OECD protocol.

**WE 020**

**The critical surface area-toxic units approach to the hazard classification of metals and alloys**

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In the search for standard, reproducible and reliable methods for the aquatic hazard classification of alloys under the GHS (Globally Harmonized System of Classification), the Critical Surface Area-Toxic Units (CSA-TU) approach offers a dependable way to derive acute aquatic hazard classification proposals based on laboratory T/D (Transformation/Dissolution) data. The CSA is the surface area loading of a substance, usually a metal, alloy or sparingly soluble inorganic metal compound, to an aquatic environment, that delivers a toxic effect. The speciation and toxicity of these substances are compared to acute and chronic ERVs. In this presentation, we use worked examples to show how the CSA- TU approach can be applied to derive this GHS hazard classification proposals for nickel-silver, iron-nickel, ferronickel, Monel, brass and Inconel alloys, and Ni and Co metals. To derive an acute hazard classification proposal for nickel-silver alloy from the pH 6 T/D data, we compared the 168-hr concentrations with a relevant ERV among 29, 120 and 70 µg/L for Cu, Ni and Zn, respectively, but because of the disparities in the reactivities of the alloy components and in the ERVs, it was difficult to select one 168-hr concentration and molecular weight conversions. To facilitate read-across to sparingly soluble metal substances, it may become necessary to investigate the rate and extent to which these compounds can produce soluble ionic and other metal bearing species in aqueous media according to OECD Series No. 290 (2001). A test system that meets the specifications of this OECD protocol has been successfully implemented to examine the transformation/dissolution behaviour of a range of metal substances, including redox-sensitive metals. The aqueous test media are based on reconstituted water prepared according to ISO 6341 with pH values in the range of 5.5 – 8.5. The OECD protocol requires exact and comprehensible procedures (e.g. accurate loadings, temperature monitorability, performance of mass balance at test end, and tests were performed in accordance with GLP requirements. In addition to a strict execution of the experimental test procedure, methods for the quantification of dissolved metal ions and species were developed and applied. Element-specific determinations by ICP-OES or ICP-MS were used and validated by appropriate QA/QC measures, including the analysis of certified reference materials (CRM) and blanks. When different methods were tested, a dedicated coupling methods, i.e. liquid chromatography coupled to ICP-MS, were developed and successfully applied to separate metal species in different oxidation states. Due to redox equilibrium processes, the stabilization of the formed redox-species immediately after sampling was critical. Differences in transformation/dissolution were observed in response to varying loadings, solution pHs, exposure times and particle surface areas. Our results found a test system to be suitable with sufficient replication as well as the benefits of testing samples with narrow particle size distributions. The relevance of these findings for read-across and the respective classification and labelling will be discussed as well as strengths and weaknesses of the current OECD protocol.
showed significantly different dissolution rates for lead. For massive lead, wire samples were mounted in epoxy resin and submitted to simulated environmental conditions of aging for 28 days before the initiation of TDp testing in the OECD media. The aging process included 3 cycles of 30-minute watering per day at 20°C under a 16/8-hour light/dark cycle. Lead, the soft metal, for polishing the surface was challenging due to implantation of polishing materials into metal matrix. A method consisting of grinding steps followed by chemical etching (77% glacial acetic acid & 25% hydrogen peroxide) was developed to overcome this problem. Interestingly, the aged and non-aged samples showed similar dissolution of lead for 7d and 28d time courses, suggesting that the corrosion layer formed during aging was labile. Additionally, the application of TICKET-UWM model successfully demonstrated the rapid removal of lead from the water column (> 70% in 28 d) and insignificant remobilization from anoxic and oxic sediments, proving compliance with rapid degradation under the GHS. This paper primarily presents TDp studies with brief information on the environmental hazard classification for lead.

**WE 022**

**Transformation/dissolution of nickel metal and sparingly soluble nickel compounds**

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T/D testing can be used to derive appropriate hazard classifications under the Globally Harmonized System of Classifying and Labeling Chemicals (GHS) and the Regulation (EC) No 1272/2008 on Classification, Labeling and Packaging of substances and mixtures (CLP). Several nickel substances underwent T/D testing in the context of REACH and GHS to evaluate the extent of transformation and dissolution in order to determine the appropriate hazard classifications. Results were compared to the existing harmonized classifications reported in the 1st ATP of the GHS regulation. T/D testing indicated that in some instances the harmonized classifications were appropriate, while in others, the harmonized classifications were either over- or under-protective compared to the appropriate hazard classification derived from the T/D data.

**WE 023**

**Classification methodology for metal concentrates**

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The application of the EU CLP regulation on single substances or mixtures of pre-defined amounts of single substances, has proven to be relatively straightforward. This is, however, not necessarily the case for complex compositions of naturally occurring substances. Metal ores and concentrates mainly contain the mineral of commercial interest, but may also hold small quantities of impurities that could trigger classification. Applying a methodology that accurately takes into account the presence of such classified impurities (e.g., Pb) as a challenge as these substances are predominantly incorporated in a metal matrix with limited bioavailability. Because of this very important bioavailability aspect, any classification based simply on the elemental composition of an ore and concentrate may generate a significantly over-stringent classification.

This presentation elaborates on the impact of different methodological strategies on the final outcome of a classification exercise, using molybdenite concentrates as an example.

It can be concluded that due to the complex, mineralogical composition of metal ores and concentrates a simplistic, elemental-based classification approach should be avoided as it assumes maximum bioavailability for each element that is present in the concentrate. The mineralogical approach, on the other hand, is based on the classification of each mineral, and a more refined and realistic classification can be obtained if bioavailability data are available for the individual minerals (e.g., T/D data for specific minerals). Bioavailability data may justify the removal or modification of a classification for a specific mineral. By conducting a T/D test on the metal concentrate, a complete picture of the bioavailable fraction of each element can be obtained, and this information can be used to derive a relevant and realistic classification of the concentrate.

**RA08P - Escape from the Ivory Tower – Environmental sciences should impact public and policy**

**WE 025**

**Science journalism - how to raise public awareness of environmental problems?**

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Communication of results is a central aspect of scientific work. However, scientists exchange their ideas mainly between their specific communities. This has, of course, advantages for maintaining in-depth discussions about particular scientific problems, but disadvantages when we think about how to communicate our results to the open public. The most important method of communicating scientific results is through publications in scientific journals. However, the feedback in mainstream mass media to such publications is very poor. Hence, once scientists are interested to increase their visibility they should think about appropriate publication formats.

Even though the field of “science journalism” has emerged over the last decade there is still a gap between the scientific community and the open public. One of the main reasons is that, based on a survey of the U.S. National Science Foundation in 2001, more than 90% of scientists think that journalists do not understand the nature of scientific research. Surprisingly, almost 80% of journalists agree. The solution of such a problem requires a long-term strategy and central aspects of such a strategy could be: (i) the education of the next generation of scientists through implementation of “science journalism” courses directly into the study programs of the natural sciences (such as the environmental sciences); (ii) the establishment of new formats for the dissemination of knowledge such as an European University TV Channel as Internet channel and as TV broadcast, which will be fed by the Media Departments of the participating Universities. A pilot project is currently running (http://eutv-university.eu/).

This would open new job perspectives for the students in different fields such as the mass media or research institutions (e.g., the German Research Foundation offers the opportunity to apply for subprojects on public relation in the bigger funding schemes such as the Collaborative Research Centres), and would offer new publication opportunities for scientists. In the long run, such a strategy should increase the quality of scientific reports in the mass media.

**WE 026**

**Establishment of a transparent ethical code for scientific communication and publication**

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In terms of “publish or perish” and “struggle for funding” scientists are under extreme pressure. Scientific communication and publication exerts a key-role in the life of a scientist, however clear and transparent standards on European or even global level do not exist. When environmental scientists communicate their work to the public they should recognize that their ‘language’ might not be readily understood outside the community were they were fed by the Media Departments of the participating Universities. A pilot project is currently running (http://eutv-university.eu)/.

This paper primarily presents TDp studies with brief information on the environmental hazard classification for lead.

**WE 027**

**How to relate information effects in environmental communication to specific audiences in order to generate maximum impact**

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When environmental scientists communicate their work to the public they should recognize that their ‘language’ might not be readily understood outside the community were it should generate impact for environmental health. This problem is known as the ‘foreign correspondent issue’ and can be taken into account by means of audience-specific language and content.

During daily work environmental scientists not only use a multitude of field-related terms when, e.g., talking to colleagues, writing manuscripts or reading literature, but they also think in a way clearly influenced by their occupation. Within the community, this ‘language’ is readily understandable and, moreover, a key to effective communication. However, when scientific findings leave the ivory tower, they require translation into common words in order to be easily perceived by the public and generate impact. But it might not be sufficient to just find regular words for technical terms. Equally important is to consider, e.g., the different knowledge, interests, opinions and desires of the various possible recipients - to put it short: the respective audience’s culture.

This presentation aims to identify the most relevant audiences for research results from environmental sciences, their key characteristics and their possible function within science communication. We will address the questions: At whom to aim? Whom to reach? How do we think? What do we seek for?

**WE 028**

**Keys to smart home diffusion: a stated preference analysis of smart meters, photovoltaic generation, and electric/hybrid vehicles - suggestions for LCA applications from the field of economics**

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Awareness of global environmental problems and the requirements for renewable energy utilization are increasing. In light of the March 11 earthquake and the Fukushima nuclear crisis, a radical reconsideration of Japanese energy policy is now being discussed. Given these circumstances, the development of a smart grid in electricity systems has raised hopes of meeting goals for climate change, energy competition, and the safety of systems and technology. This leads to many economic questions related to incentive policies for the implementation of such technology.

Smart grids can be classified into two systems: the upstream power supply system and the downstream power demand system. This paper deals with the latter, specifically concerning residential demand for smart equipment to make up smart homes. In particular, we investigate the future diffusion of advanced or smart meters (SM) that measure electricity consumption hourly, photovoltaic (PV) generators that are installed on residential rooftops, clean-fuel electric vehicles (EV), and hybrid electric vehicles (HEV). The implementation of these technologies in Japan is imminent, and policy is needed to prepare for smart home diffusion. This paper will conduct a conjoint analysis of the future diffusion of SM, PV, EV, and HEV using the results of an online survey administered in March 2011. Through this estimation of consumer future demand and expected greenhouse gas emissions, this paper suggested a connection between economical demand analysis and LCA research.

**WE 029**

**PRIMO’s next - advanced student projects on the fundamentals of science, environment and health**

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"Biomarkers are the answer! But what is the question?" After many ecotoxicology conferences we had the slight sensation that students were lacking creativity and losing the ability of asking good questions.

To discuss these premises, we organized 2 editions of the ‘International School on Marine Ecology and Ecotoxicology’, now called PRIMO’s Next, gathering a group of 30 students and 15 instructors from 15 different countries, for 10 days in October 2010 and 2011, in Brazil.

The result was outstanding! The high level of technical classes and discussions about science activity and communication invited the students to think about their own work, from their own laboratories to the communication and the proposal of the results, in a way they never did before.

The significant improvement on their presentations can be observed comparing the platform presentation in this section from students that attended the school with the students’ seminars videos recorded at the school and available on youtube.

From now on, we are proposing to organize PRIMO’s Next’ meeting aiming to prepare the next generation of PRIMO attendees to make PRIMO better and better. This ‘school’ would take place every two years, in different developing countries, in between PRIMO meetings.

**WE 030**

**A holistic approach for improving academic teaching and education**

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\(^4\)High School Students Lab “Fascinating Environment”, which was established at RWTH Aachen University in 2009. The students lab was founded to strengthen education and training in environmental sciences by providing state-of-the-art analytical and bioanalytical facilities and meanwhile has matured into a holistic approach to academic teaching.

Most of the lab equipment became available thanks to sponsoring by more than a dozen industry partners. The idea behind the cooperation with the universities was to provide a university platform for interested companies to present their products, whereas simultaneously benefitting from the utility of sponsored equipment for education and research. This approach has resulted in a large number of bachelor and master theses and also several international peer-reviewed scientific publications. The students lab furthermore maintains a close cooperation with the Institute for Teaching Methodology in Biology at the RWTH Aachen University. Several school adaptations of ecotoxicological test systems are currently being developed by future teachers with the aid of the students lab to sustainably integrate ecotoxicology into the curriculum of secondary school-education and early interest pupils in environmental sciences. A recent project in the students lab has adapted the fish embryo test (FET) with the zebrafish to a short assay of the development of aquatic test organisms on how to use, conduct the test directly at schools, portrays special project days that take place at the students lab on a regular basis and includes a general introduction into work at an academic institution.

As of winter term 2010/11 the new project FILM started in association with the students lab. The aims of FILM are (a) to capture scientific results on film, (b) to plan and produce short movies about scientific test systems, (c) to process both for a broad variety of possible audiences and (d) to comprehensively and unambiguously present one’s own research in a filmed interview situation. The project was included into an already existing skill course system for students of biology and ecotoxicology.

**WE 031**

**Developing entrepreneurship in Russian environmental science**

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Problem of effective communication between “Ivory tower” scientists on the one hand and public, business and policy-makers on the other hand is a barrier for sustainable development of Russia. National society has the capacity to develop effective environmental technologies and solutions which could provide the opportunities to achieve “win-win” results: allows to get both environmental and economic effects. After the collapse of the USSR, process of transfer of research-based innovations from academia to economy suffered serious decline and nowadays implementation of innovations. There is a need of stimulating of entrepreneurial mindset among academics and students, student involvement through entrepreneurship education programs, developing infrastructure, incubators and government support programs.

There are several hypotheses, which concern the opportunities for entrepreneurship in environmental science and promotion of cleantech innovations in Russia.

- Russian scientific potential has potential to develop cleantech innovations, primarily, in natural sciences that were competitive during the Soviet times, including biology, chemistry and physics.

- Natural science professionals, who could develop innovations don’t usually possess entrepreneurship and management skills to commercialize it.

- There is an apparent lack of infrastructure for transfer of innovations, lack of number in business incubators, technology offices, legislation and laws do not provide the sufficient level of intellectual property protection.

- Experiences of the past show that government played key role in development of innovations. Nowadays, innovations mostly associated with ineffectual budget money distribution due to political goals, rather than from economic and scientific goals.

- Scientists have lack of motivation to participate in transfer of technologies, which were developed in governmental organizations since scientists would not get sufficient financial reward.

- Potential future innovators and inventors, who study in Russia would rather go to the western countries, where more appropriate environment for the development.

- There no appropriate education and learning opportunities for entrepreneurs who can participate in transfer of knowledge.

**WE 032**

**Sustainable training concept on chemicals risk assessment in Serbia**

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The Serbian Chemical Agency (SHemA), as competent authority for chemicals management on national level, plays a crucial role in the establishment and enforcement of the legal framework in this field. Capacity building in order to prepare SHemA staff for responsibilities within the European chemicals legislation is imperative. Currently, SHemA has potential to develop such training, with the help of the Twinning project and project implemented by Swedish Chemicals Agency (KeMl). The objective is to support the successful implementation of the national legislation on chemicals management and thus contributing to the protection of human health and of environment by improved practices for the safe use of chemicals and biocides in Serbia. The project is one of the most intensive trainings are carried out towards lecturers with practical exercises, trainings aimed as a next step to apply approach of learning-by-doing and training-on-the-job. In order to create a modern interdisciplinary universities’ program related to chemical safety, with special accent on chemicals risk assessment provided within the present IPA 2007.

**WE 033**

**Risk and uncertainty: bioavailability based environmental quality standards for metals**

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The Environmental Agency for England and Wales, the Environment Agency, is in the process of redefining environmental quality standards (EQSs) for copper and zinc in terms of a permissible bioavailable concentration value. Whereas the consideration of bioavailability improves the ecological relevance of EQSs, assessing the extent by which compliance with EQSs expressed in terms of bioavailability is achieved is complex since bioavailability cannot be measured directly and requires tools, such as the Biotic Logat Model (BLM), to convert the bioavailable value into a measurable dissolved concentration. BLMs require, as a minimum, pH, dissolved organic carbon (DOC) and inorganic complexation inputs, however, concentrations of DOC are at times variable and consequently, there is uncertainty in the extent by which water bodies in England and Wales are likely to comply with future standards. Compliance is important, not least of all because it provides an indication of environmental risk, but also because it informs decisions on the degree to which sewage effluents are required to be treated. Since the management of sewage treatment capacity requires a long planning horizon, it is useful in advance, in the impact of new regulatory regimes in order to facilitate planning. However, for the reasons given above, this has only been possible at a limited scale. In order to quantify the potential impact of bioavailability based standards on compliance, a Monte Carlo approach...
was applied to generate a distribution of EQS values for each water quality monitoring location in England and Wales based on probable combinations of pH, DOC and Ca. For sites that were missing water chemistry data the characteristics of its nearest neighbour for which data was available were applied. The results indicate that, at an aggregate level, the absolute number of monitoring locations at risk of failing to comply with the EQSs as they may become, was unlikely to change, however, a redistribution of locations at risk appears likely and suggests that, from a water company perspective, there are likely to be winners and losers. The risk assessment results and an overview of the stakeholder responses to the results and the approach are provided.

WE 034
Old news or real risks? Bisphenol A under scrutiny
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Having the daily news on Bisphenol A (BPA) in mind, it becomes clear that this substance still rises concern and seems to be a never ending story - scientifically as well as politically. BPA substances such as plastic containers made of polycarbonate, water pipelines made of epoxy resins or cash receipts made of thermal paper. Environmental compartments may be exposed via sewage water treatment plant effluents from paper recycling, water pipelines, industrial processes or by leaching from plastic products made of polycarbonate. It is used wide dispersively and is produced with a high tonnage of many manufacturers in Europe. Despite the huge amount of generated data and accomplished risk assessments for BPA, it is still controversially discussed how to assess this substance. It is well known for its endocrine effects on aquatic organisms at very low concentrations. However, there are different interpretations of animal tests and disagreements on the assessment of its endocrine activity. Additionally, some monitoring studies indicate that environmental concentrations might be higher than expected based on existing data. It is questioned whether there is a correlation to the health concerns and how other substances contribute to make a complex environmental exposure.

Because of these uncertainties the question rises how to soundly regulate Bisphenol A. UBA proposed a comprehensive substance evaluation under REACH in order to clarify if more or other data is needed and how Bisphenol A should be regulated in future. Possible consequences might be a restriction of certain uses or the identification of BPA as substance of concern due to its endocrine disrupting properties.

This presentation aims at giving a structured overview on the challenges mentioned and the steps towards a solution for Bisphenol A from an environmental perspective.

WE 035
Management of risks of very high concern: first challenges with the authorisation process under REACH
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The Authorisation process is one of the major Risk Management Options for Authorities under REACH. This is a three step process:
- Identification of Substances of Very High Concern (SVHC, started 2008)
- Inclusion of Substances in Annex XIV, the Authorisation List (first recommendation in 2009, first Annex XIV beginning 2011)

The poster will present objectives, some statistics and challenges faced during the first years of SVHC identification and inclusion of substances in Annex XIV.

The list is designed as a tool to facilitate the consultation of information about 1,100 substances of very high concern for the environment and human health. The list is periodically updated and they include "Activities and documents", "Databases", "Information for the public", "European and national legislation", "Substances identified as SVHCs and included in the Candidate List, are prioritised and recommended for inclusion in Annex XIV by ECHA. The REACH regulation outlines three criteria for priority setting in Art. 58(3):

- PBT or vPvB properties;
- Wide dispersive use;
- High volumes.

ECHA and the MSC, which gives its opinion to ECHA's proposal, agreed that regulatory effectiveness needs also to be considered. Each of these criteria has its issues that ECHA take into account in a two tiered approach, a scoring exercise and an argumentative approach.

The wide dispersivity has been broken up in two sub criteria, wide spread uses and the possibility of releases. This does not aim to evaluate risk as such, but the potential for exposure.

The main issue with the volume is identified in the volume in the scope of authorisation requirement, as others are not taken into account.

- Regulatory effectiveness is particularly important for groups of substances with same hazard or with UVCB substances.

WE 036
Timber expert system as information platform and communication channel for innovative and widely applicable strategies, technologies and solutions for contaminated sites risk assessment
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Risk-based approach for sustainable management of contaminated sites and brownfields produced in the last years an abundance of regulations, strategies, guidelines, tools, documented case studies, assessment methodologies, as well as risk communication methodologies and tools, which are too often not used in their entire potential. The non-visibility of already available, useful and innovative methodologies and tools for environmental risk assessment and in general for sustainable management of contaminated sites is a challenge for stakeholders, who are often not aware of the existing tools and methodologies because of a lack of visibility of already available, useful and innovative methodologies and tools for environmental risk assessment.

The "7th Framework Programme project timbre (timber improvement for brownfield regeneration in Europe) aims at providing contaminated sites’ owners, local authorities and stakeholders with a web-based expert system that can be at the same time a user-friendly collector and provider of all available information related to environmental risk assessment and sustainable management of contaminated sites for experts, stakeholders and decision makers involved in the risk-based management of contaminated sites and brownfields. The first step for the development of the timber expert system consisted in a shared decisional framework for the assessment and sustainable management of contaminated sites and brownfields which is composed of the main phases of the risk-based regeneration process (e.g. site characterization phase, environmental risk assessment and communication phases, etc.). The process to develop the framework has been enriched with a strong collaboration with the timber case studies stakeholders who have been involved in the rehabilitation framework development through a participatory process which comprised the organisation of local workshops and the filling in of an appropriate questionnaire for evaluation of the contaminated sites regeneration framework. The active involvement of stakeholders represents a strong guarantee that the scientific process will produce useful and shared results, which will be properly communicated between all the actors involved in the risk-based contaminated sites and brownfields regeneration process.

WE 037
REACH and other regulations: information and communication on chemicals
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For the implementation of the REACH Regulation (Registration, Evaluation and Authorisation of Chemicals), the Italian Ministry of the Environment, Land and Sea has the task to give information about the risks of chemicals. Among the initiatives promoted by the Ministry, we highlight the e-bulletin “Sostanze chimiche - Ambiente e Salute”(“Chemicals - Environment and Health), the “Lista delle sostanze violate (o in restrizione)” - (List of banned or restricted substances) and the Ministry’s official website.

1. The e-bulletin deals with activities and news related to REACH, other Regulations concerning chemicals and risk mitigation measures. The aim of this newsletter is to provide, through a simple language, adequate information for the public on substances/biochemicals.

The e-bulletins already have been presented at the 3rd National Conference on REACH, which was held in Rome on November 14, 2011.

2. The e-bulletin is available at www.minambiente.it and it’s possible to receive it sending an e-mail to sostanzechechimiche@minambiente.it.

3. The list of substances provides a picture of substances banned, restricted or subject to the authorization procedure under Regulation (CE) n.1907/2006 (REACH). The list is designed as a tool to facilitate the consultation of information about 1,100 substances of very high concern for the environment and human health. The list is currently available on the Ministry’s website.

4. The Ministry’s website contains two sections “REACH” and “Environmental risk assessment” where it’s possible to find information about chemicals under REACH Regulation, Plant Protection Products and Biocides.

Information on REACH
http://www.minambiente.it/home_it/menu.html?m=menu_attivita&c=P=REACH.html&lang=it

Information on “Environmental risk assessment”:
http://www.minambiente.it/home_it/menu.html?m=menu_attivita&c=argomenti.html|Valutazione_del_Rischio_Ambientale__VRA_.html&lang=it

These sections are periodically updated and they include “Activities and documents”, “Databases”, “Information for the public”, “European and national legislation”, “Events” and information from “European Chemicals Agency (ECHA)”.

These initiatives address:
- General public
- Environmental and consumer organizations
Hazard and risk assessment of chemicals is a highly complex task, because there are thousands of chemicals and a wide range of effects in numerous species that need to be assessed. Therefore, careful evaluation and interpretation of both the available scientific knowledge and the existing data gaps and uncertainties is essential. This evaluation and interpretation should be focused on the needs of decision makers outside the scientific field. Because every single chemical may pose highly complex questions for the assessment of exposure and effects, additional experts are called as individuals and/or representatives of institutions working at this interface, the International Panel on Chemical Pollution, IPCP. The IPCP focuses on chemical pollution problems of global relevance and is a platform where academic scientists can jointly evaluate their findings and collaborate on the transfer of these findings to decision makers. In several existing interface institutions in the field of chemical assessment, scientists are called as individuals and/or representatives of institutions working together in these interface institutions. Taking a different approach, the IPCP is a platform primarily for academic scientists who want to share their knowledge about certain aspects of chemical pollution problems and want to join a global network that supports them in conveying their knowledge to decision makers at the national and international level. The objective is to provide an overview of the current activities of the IPCP with a focus on communication of scientific results to the public.

PREPARE knowledge exchange initiative: Pharmaceutical Release into the Environment under Pandemic and Regional Epidemics

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3PREPARE is a Natural Environment Research Council-funded Knowledge Exchange Initiative which is a multi-disciplinary network aimed at providing a foundation of scientific understanding on the fate and effect of widespread pharmaceutical use during a pandemic and epidemic. By bringing together industry and the research community, it is anticipated that the Initiative partners will contribute to a thorough assessment of the risks, a more thorough assessment of the known-knowns and an informed determination of the knowledge gaps. It is the aim of PREPARE to not only identify novel hazards but also to spark innovative solutions and coordinate the research to fill knowledge gaps.

WILDCOMS

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Disease and contaminants can both pose major risks to wildlife and human populations. Disease is a natural driver regulating the dynamics of wildlife populations, but some diseases warrant particular attention because they (i) cause major mortalities that lead to population crashes, (eg, VHD in rabbits), (ii) threaten wildlife species of high conservation concern (for example squirrelpox virus in red squirrels), or (iii) pose a potential threat to Man (eg, rabies, avian influenza). The wildlife Disease & Contaminant Monitoring and Surveillance (WILDCOMS) Network is a collaborative project among the major disease and contaminant monitoring schemes for vertebrate wildlife that operate in the United Kingdom. These schemes are run by various government agencies and laboratories, research centres, institutes, and academia. The overall aim is to establish a network which will foster and facilitate knowledge exchange, harmonisation towards best practice, and productive collaboration between (i) partner organisations; (ii) surveillance schemes and end-users. It will aim to provide end-users with an integrated overview of environmental disease and contaminant risk. The specific objectives will be to develop the network and use it to address common challenges, specifically maximising dissemination of information to stakeholders and harmonisation towards common operational procedures to facilitate interaction and collaboration.

PREPARE

Nyabondo integrated vector management project

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Malaria remains a major vector borne disease in many countries in sub-Saharan Africa. This is true for Kenya where an integrated vector management program was implemented on Nyabondo plateau to manage mosquito populations with the main goal of interrupting malaria transmission. Malaria mosquitoes spend a considerable part of the pre- and post-oviposition stages in water that are not suitable, hence vectors are not able to complete their life cycle and therefore the habitable areas for these mosquito species remain limited. This activity leaves behind numerous pits in which water accumulates to create conducive mosquito breeding habitats. Abandoned fish ponds add to this to aggravate the mosquito problem on the plateau. We combined the biological larvicide Bacillus thuringiensis israelensis (Bti) and introduced mosquito-eating fish Oreochromis niloticus in abandoned ponds to test its effectiveness for mosquito control in Nyabondo. In addition, because most of these habitats are man-made the involvement of community members through capacity building and knowledge transfer has been implemented as a critical part to ensure sustainability of malaria control measures. Application of Bti within breeding habitats reduced 86.3% of larvae by day 1 and 95% by day 4 when compared with day 0 i.e before Bti was applied. There was a 100% reduction in mosquito larvae within active (stocked with fish) ponds when compared to un-stocked fish ponds (abandoned). Mosquito vector control needs complementary strategies that target both the adult and larval stages. The two main habitats mentioned (brick pits and abandoned ponds) are as a result of human activities and therefore the project will continue to encourage and involve the community in the control measures.

Risk perception analysis of the use of pesticides in rural areas: a study with farmers in Bom Repouso (Brazil)

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Bom Repouso is localized in Minas Gerais State, Brazil and approximately 50% of its population resides in rural areas, producing potato and strawberry in large scale to support the national demand. However, the intensive use of pesticides by farmers has resulted in undesirable effects after 20 years. How notified by the farmers, cancer and suicide cases are increasing in this region, as such as the environmental problems. Therefore, our hypothesis is that the exposure to pesticides could be recognized as an important environmental risk factor, but farmers underestimate the consequences. Then, we tried to understand how farmers think about and respond to risk in relation to the use of agricultural pesticides and the possible consequences. The results showed up the extension of agricultural seminars; field studies (2009-2010) to identification of the Preliminary Assessment of Risk and interview with 50 farmers (selected among the 22 neighborhoods from the rural area), assessing their perception about risks associated to the current production model. Preterit data (obtained in 2005-2008) were also incorporated in the research, which allow us to recognize the social economical profile of the 1480 farmers and information about local structure. The results showed up the expansion of the agriculture, of chemicals assessment, scientists are called in as individuals and/or representatives of institutions working at this interface, the International Panel on Chemical Pollution, IPCP. The IPCP focuses on chemical pollution problems of global relevance and is a platform where academic scientists can jointly evaluate their findings and collaborate on the transfer of these findings to decision makers. In several existing interface institutions in the field of chemical assessment, scientists are called as individuals and/or representatives of institutions working together in these interface institutions. Taking a different approach, the IPCP is a platform primarily for academic scientists who want to share their knowledge about certain aspects of chemical pollution problems and want to join a global network that supports them in conveying their knowledge to decision makers at the national and international level. The objective is to provide an overview of the current activities of the IPCP with a focus on communication of scientific results to the public.
WE 043 Needs and opinions on risk regulation from Swedish companies using nanomaterials
L. Schenk
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Nanotechnologies are emerging technologies, still diversifying in many scientific fields and industrial sectors. Regulatory concerns regarding the use of nanotechnologies and more specifically nanomaterials originate in lack of knowledge regarding their use and potential effects. The nanoscale properties that make nanomaterials so appealing for the companies as well as the potential of the nanoscale substance most likely will also change compared to the bulk material. It is important to consider future applications of nanotechnologies as well as current when discussing regulatory needs and actions.

In an overview of research and development activities within Sweden, a questionnaire was sent out to nanotechnology companies. The questions covered areas such as use of nanomaterials, risk assessment efforts and the companies’ views on the use of nanomaterials, business conditions and how governmental agencies should work with such issues. Previous overviews suggest that close to 120 companies are active within nanotechnology in Sweden. By using a broad set of criteria 176 companies were contacted for the purpose of this study, of which 35 responded that they were not active within nanotechnology and 48 responded that they were and filled in the questionnaire. The latter were represented by four out of every five states that they were active, which according to the companies was seen by the companies as an obstruction to a successful innovation climate in Sweden. The results from this questionnaire will be presented and discussed in relation the needs of regulation and communication efforts.

WE 044 Are labeled products safer?
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Around 2-4% of the German population suffer from contact allergy to fragrance compounds. In the EU, 26 supposedly allergic fragrances must be specified on the containers of cosmetic products and washing and cleaning products if they contain certain mass percentages in the product. Three parties (manufacturers, authorities and consumers) carry the responsibility in this kind of risk reduction strategy:
- Manufacturers must specify the "26 allergens" on the label of their products.
- Authorities should control the implementation of the labelling, at least by spot checks of the e.g. around 20 000 new or modified cosmetic products that enter the German market every year.
- Consumers carry an even bigger share: They should read the label, know which names belong to the "26 allergens" and decide whether they want to buy and use this product containing potential allergens. Consumers need to know a lot, they should be able to make a kind of risk assessment for their private use and decide whether they want to take the risk or not. On top of that, most consumers are not aware that they carry this responsibility and assume that cosmetic products were free of hazardous substances. The labelling of the "26 allergens" is a risk communication instrument. However, it does not communicate the unavoidable "residual" risk after all feasible risk control measures have been taken. It rather changes the general public with the tasks of risk assessment, risk control and risk management, even if their risk perception is minute or even nonexistent.

Although the cosmetics industry takes over more responsibility for the use of allergenic fragrances by this "26 allergens rule" than producers of other products where no labeling is required, a mandatory labelling procedure is not suitable to guarantee consumer safety. Products need not be safer now than before the compulsory labelling, unless consumers exert their power and would no longer buy products where these fragrances are labeled, which is apparently not the case in the German market of deodorants.

Experts elaborating criteria for labelling products containing nanomaterials as risk reduction strategy could learn from the experiences made here. It must be questioned whether such a compulsory labelling alone is a suitable way for risk communication and management.

WE 045 Practical guidance for undertaking socio-economic analysis within the REACH authorisation process

The sensitivity model is carried out by bringing the stakeholders from the region of concern together and by letting them work together on a system description of that area. Different perceptions and various levels of knowledge lead to a high level of discussion among the group. The group needs to agree on the system description, sharing a lot of information about possible alternatives, information about the impacts if authorisation is refused. A Chemical Safety Assessment (CSA) conducted under REACH will not contain sufficient information to conduct an SEA. An SEA will take time, so it is best to plan ahead even if external help is used. Information will need to be gathered within organizations and from suppliers and customers, potentially from other manufacturers (competitors) and stakeholders. Frameworks for sharing information, whilst ensuring confidentiality and competition laws are respected, will need to be established. Perhaps most importantly, SEA should be viewed as an opportunity to demonstrate the value of a product to society.

WE 046 SIMACLIM - a combination of the regional risk model and a communication tool
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SIMACLIM consists of two parts: A sensitivity model according to Vester and a relative risk model which is based on the regional risk model by Landis and Wiegner (1997). The sensitivity model is carried out by bringing the stakeholders from the region of concern together and by letting them work together on a system description of that area. Different perceptions and various levels of knowledge lead to a high level of discussion among the group. The group needs to agree on the system description, sharing a lot of knowledge while doing this, building up trust among each other. Part of the project is also to address and agree upon management objectives in the region. These management objectives represent the input from the sensitivity model to the relative risk ranking model. The latter is intended to be used by decision makers to show the decision makers to the stakeholders the various state of the environment.

These decision makers could also imply that the importance of size is overemphasised, both by media and regulators. This together with perceived risks of regulatory actions could be seen by the companies as an obstruction to a successful innovation climate in Sweden. The results from this questionnaire will be presented and discussed in relation the needs of regulation and communication efforts.

WE 047 Selection of potential focal bird species for a refined risk assessment of pesticides in Switzerland: methodology and examples
M. Gandolfini, T.S. Reichlin
Study Station Agroscope Changins-Wädenswil ACW, Wädenswil, Switzerland

Pesticides are subjected to ecotoxicological risk assessments in the process of national authorization. If a pesticide does not pass the first-tier, worst-case risk assessment, a detailed or second-tier risk assessment is required. For birds, it is possible to refine the exposure component of the risk assessment for first-tier species, real bird species actually occurring and feeding in the crop at the time of pesticide application. Therefore, using focal species, realism can be added to the risk assessment. The potential focal bird species for Switzerland for the main Swiss cultures were selected with a semi-quantitative method based on comprehensive Swiss ornithological literature and on expert knowledge of Swiss ornithologists. All candidate bird species were judged independently by different experts with regard to their suitability as focal species. The key criteria were i) strong association to the crop, ii) high abundance in the crop, and iii) high intake of contaminated food per kg body weight. For each main culture and growth stage, two to four focal species with different feeding habits were selected. In this presentation, the methodology behind the selection of focal species will be described, together with some concrete examples of the potential Swiss focal bird species. These focal species could be used in the future to achieve a crop-specific and more realistic estimation of the risks of pesticides in Switzerland.

WE 050 List of potential focal bird species for a refined risk assessment of pesticides in Switzerland
M. Gandolfini, T.S. Reichlin

List of potential focal bird species for a refined risk assessment of pesticides in Switzerland
If a pesticide does not pass the first step of the ecotoxicological risk assessment for birds, a further refined risk assessment is required. This involves the use of more realistic exposure estimates and the use of “focal species”. These species are, other than the fictitious first-tier species, real bird species actually occurring and feeding in the crop at the time of pesticide application in the field. Therefore, using focal species, realism can be added to the risk assessment. The focal bird species potentially relevant for Switzerland were determined by the ecotoxicology group at the Research Station Agroscope Changins-Wädenswil (ACW) based on comprehensive Swiss ornithological literature, with the helpful input from experts of the Swiss Association for the Protection of Birds (SW/BirdLife) and the Swiss Ornithological Institute Sempach. The suitability of bird species as focal species is mainly based on the probability of their being present in the crop and their sensitivity to the specific chemical. The key criteria were: i) strong association to the crop (intensive access and utilization), ii) high abundance in the crop (high frequency and distribution), and iii) high intake of contaminated food per kg body weight. For each main Swiss culture and growth stage, two to four focal species with different feeding habits were selected. In this poster, the selection procedure and the whole list of the identified focal species is presented.

WE 051 Selection of focal species of birds and related exposure scenarios for different crops treated with pesticides in Norway

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The work presented here is the result of a six-year project to select Norwegian focal species financed by the Norwegian Ministry of Agriculture and Food. At the initiation of the study, the review covering a number of species that utilize the Norwegian farmland landscape, and which species might be susceptible to pesticide exposure was conducted (Bakken & Strøm 2006). The method was based on a Swedish approach for selecting focal species (Wärmback 2006). Field surveys aimed at selecting focal species for strawberries were conducted as a pilot study during the 2007 growing season (Bakken et al. 2007) and field surveys in eight fields of different crops were conducted in 2009, resulting in work being conducted in 2009 in 8 new fields of the same crops. In 2011, ten fields of five new crops were surveyed. The Norwegian field surveys followed the repeated line transect method. All birds sitting, taking off from, or landing in the fields, were recorded. The birds where identified, either directly or with the aid of binoculars. Approximately 130 hours of field work was conducted between May and October each year. A total of 38 different species of birds were identified in 2008, 38 species in 2010, and 33 species in 2011.

In order to identify focal species, the frequency of observation per survey (FSurveys) was calculated for each species. FSurveys denotes the number of surveys in which a defined species was recorded given as a percentage of the total number of surveys (EFSA 2009). This gives an approximation for the temporal consistency of occurrence throughout the study period and gives an indication of prevalence. From our field surveys, along with an evaluation of published data, the Norwegian Food Safety Authority has identified a list of focal species and related scenarios for the following 12 crops/groups of crops: grassland, cereals, oilseed rape, strawberries, pulses, potato, orchards, bulbs and onion like crops, bush and cane fruit, leafy vegetables, maize, and root and stem vegetables.

WE 052 Focal bird species for non-agricultural ‘Grassland Scenario’ risk assessments

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In the new EFSA guideline for risk assessments for birds and mammals a list of crop groups is given to identify relevant scenarios for wildlife risk assessments. However, the scheme of crop groups given in the EFSA guidelines is not easy to apply to non-agricultural applications such as for example non-agricultural infrastructures. As a result of different habitat requirements in this field, the idea was developed to consider those communities as a common denominator for the bird community on golf courses, and were thus determined as the main candidates for focal bird species. Therefore, further field observations and surveys will be conducted on golf courses in south western Germany in spring 2012 in order to verify candidate bird species for high tier risk assessments.

Agricultural land utilization offers a variety of different habitats for wild birds. Based on the results of published bird surveys, a total of 799 breeding pairs of 88 different species, were recorded on 6 golf courses in central Europe. The blackbird, white wagtail, great tit, blue tit, greenfinch, blackcap and the chiffchaff were the most characteristic and stable elements of the bird community on golf courses, and were therefore used as the main candidates for focal bird species.

The current bird and mammal risk assessment scheme for pesticides in Europe (EFSA Guidance document, 2009) considers specified “indicator species” or “generic focal species” as worst-case species of birds and mammals to be used in risk assessment (Alauda arvensis, Emberiza citrinella, Anser fabalis, Anser anser, Perdix perdix, Motacilla flava, Parus caeruleus, Erithacus rubecula, Fringilla coelebs, Serinus serinus, Carduelis carduelis, Carduelis cannabina, Turdus merula and Sturnus vulgaris).

According to revised Guidance document on risk assessment for birds and mammals, published in EFSA Journal 2009; 7(12):1438, focal bird and mammal species used in risk assessment of Plant Protection Product (PPP) are real species that actually occur in the crop when PPP is being applied. Selected species should cover all other species from the feeding guild highlighted at the screening level on Tier 1 of risk assessment.

In our literature review we primarily focused on bird occurrence in Slovakia that can be potentially used as a focal species in risk assessment. We reviewed recently published articles or books. On the basis of the proposed scenarios in the updated guidance document published by EFSA in 2009 and our literature review, we focused on a total of 14 bird species as potential focal species to be used in risk assessment (Alauda arvensis, Emberiza citrinella, Anser fabalis, Anser anser, Perdix perdix, Motacilla flava, Parus caeruleus, Erithacus rubecula, Fringilla coelebs, Serinus serinus, Carduelis carduelis, Carduelis cannabina, Turdus merula and Sturnus vulgaris).

We suggest to add the Grey Partridge (Perdix perdix) to the list of focal species as insectivorous bird species. The chick’s diet predominantly consists of insects in the first three months of their life. Risk assessors use Grey Partridge as medium herbivorous bird and the importance of insects in the chick’s diet is not taken into account in risk assessment. Therefore if the PIP is applied from beginning of April till beginning of June, we suggest to use the Grey Partridge as an insectivorous bird and after that time period as a medium herbivorous bird species.

The use of the Yellow Wagtail (Motacilla flava) as a focal bird species in refinement of potential risk (proportion of a bird’s daily diet obtained in habitat treated with pesticide) is questionable, because it is rare occurrence in Slovakia. We suggest to replace Yellow Wagtail with Skylark or Grey Partridge (April - June), where possible.

On the basis of our literature review we fully support to use of these one species of limited value for risk assessments (Motacilla flava);

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WE 054 Shift in crop preference by some focal farmland bird species during the breeding season in The Netherlands

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European farmland bird populations have been in a steep decline since the 1960s. Agricultural intensification is one of the main factors which has driven these declines. Intensive use of pesticides is one of the many factors that is covered by agricultural intensification. Several studies have shown that pesticide use can have direct and indirect effects on bird populations. The selective success of pesticide use is important to assess in arable landscapes. Therefore, the development of the crop and associated changes in vegetation cover within an arable landscape with different crops. Pesticide applications in crops which are strongly preferred by birds are most likely to have most effects on these birds. In the first place because of possible direct effects on the birds, but also due to indirect effects as a result of reduced food availability. Therefore, results of detailed crop preference studies on birds, including procedures of pesticide application, is important.

WE 055 Bird focal species for pesticide risk assessment in orchards in Spain

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The current bird and mammal risk assessment scheme for pesticides in Europe (EFSA Guidance document, 2009) considers specified “indicator species” at the initial Screening Step and “generic focal species” at Tier 1 assessment. This “indicator species” or “generic focal species” are not real species but are considered to represent worst-case scenarios and hence be of protective to other species occurring in that crop. In order to conduct a refined risk assessment is necessary to identify “focal species” i.e. species

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that actually occur in the crop in question and that are protective of other species. Field studies were conducted in citrus plantations, olive groves and stone fruit plantations in Spain to identify the relevant bird focal species in these crops. Standard methodology for the "transect method" as detailed in the EFSA Mammal and Bovine Risk Assessment Guidance (2009) was employed. The most recent scientific regulatory guidance documents developed in order to conduct risk assessments for birds and mammals for plant protection products (PPP) for birds and mammals for plant protection products (PPP), the potential risk for the avian community is identified, apart from toxicity, on the basis also of a theoretical exposure via use of crop specific 'focal species' (FS) in various BBCH scales. The FS concept means one or group of species, having spatial and functional requirements effectively defining environmental limits for the protection of other species present in the crop. Field size, land use, its management and characteristics of the surrounding non-crop areas, crop rotations can influence the bird fauna and the way birds use the agricultural fields. It can be difficult to assess the relationship between them as the mobility of birds allows them to use the available habitat on a larger-scale than that which might be expected for such associations. The presence of focal bird species is influenced by the availability and characteristics of alternative habitat types and therefore specific crops and their bird populations do not function as isolated patches. The interrelation between non-crop, crop areas and crop types as foraging habitats for birds will be influenced by the relative size of the fields and of off-crop areas. Such interrelations may be complex in agricultural landscapes characterized by small fields of different crops with diverse off-crop areas as hedgerows or bushes between the fields. Such landscape occurs frequently in Mediterranean countries, more than in other regions of the EU. For such Mediterranean landscapes, it is necessary to obtain more detailed knowledge about bird use of different habitats before defining the most appropriate FS that can be proposed. The aim of this study is to present data on bird FS from a typical Mediterranean agricultural landscape in Hellas (Kopaida plain) with such diversity of rotational crops and off-crop habitats.

WE 056
Farmland bird communities vs crop specific focal species in a Mediterranean landscape: The case of arable crops in Hellas
M. Fouadoukakis
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In Europe, regulatory bird and mammal protection guidelines developed in order to conduct risk assessments for birds and mammals for plant protection products (PPP) require the identification of bird focal species (FS) that actually occur in the crop in question and that are protective of other species. Standard methodology for the "transect method" as detailed in the EFSA Mammal and Bovine Risk Assessment Guidance (2009) was employed. The most recent scientific regulatory guidance documents developed in order to conduct risk assessments for birds and mammals for plant protection products (PPP) for birds and mammals for plant protection products (PPP), the potential risk for the avian community is identified, apart from toxicity, on the basis also of a theoretical exposure via use of crop specific 'focal species' (FS) in various BBCH scales. The FS concept means one or group of species, having spatial and functional requirements effectively defining environmental limits for the protection of other species present in the crop. Field size, land use, its management and characteristics of the surrounding non-crop areas, crop rotations can influence the bird fauna and the way birds use the agricultural fields. It can be difficult to assess the relationship between them as the mobility of birds allows them to use the available habitat on a larger-scale than that which might be expected for such associations. The presence of focal bird species is influenced by the availability and characteristics of alternative habitat types and therefore specific crops and their bird populations do not function as isolated patches. The interrelation between non-crop, crop areas and crop types as foraging habitats for birds will be influenced by the relative size of the fields and of off-crop areas. Such interrelations may be complex in agricultural landscapes characterized by small fields of different crops with diverse off-crop areas as hedgerows or bushes between the fields. Such landscape occurs frequently in Mediterranean countries, more than in other regions of the EU. For such Mediterranean landscapes, it is necessary to obtain more detailed knowledge about bird use of different habitats before defining the most appropriate FS that can be proposed. The aim of this study is to present data on bird FS from a typical Mediterranean agricultural landscape in Hellas (Kopaida plain) with such diversity of rotational crops and off-crop habitats.

WE 057
Exposure evaluation for wild mammals in ornamental gardens in the Netherlands
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An ecotoxicological risk assessment must be performed prior to authorization for all plant protection products in the EU. For birds and wild mammals, "generic focal species" scenarios are proposed as initial steps in the Guidance Document of EFSA for "Risk Assessment of Birds and Mammals". As a second step, a refined ecotoxicological risk assessment must be conducted based on real focal species for the respective exposure scenario. For many major crops (e.g., cereals) such information is available in the literature or from targetted field studies, but little is known for a range of minor crops, such as ornamental gardens. We aimed to determine focal species in tulip and lily fields in the Netherlands by setting up a trapping grid with life traps for three trapping nights. We typically found Wood Mice in and around the field, and Bank Voles only in forested surroundings. Additionally a few Common Voles were trapped.

Investigated parameters included the qualitative composition of the bird community, the frequency of occurrence, dominance and abundance of species present. The poster will propose a list of bird FS for arable crops used as an ecological niche by birds, assigned to foraging guilds, diet guilds and size classes. Their use in a refined risk assessment for PPP is discussed further in the final poster.

WE 058
Focal species and their ecological behaviour in pesticide risk assessment in the Netherlands
J. Wassenberg
J . Wassenberg
Focal species and their ecological behaviour in pesticide risk assessment in the Netherlands

WE 059
Risk assessment for birds and mammals in Mediterranean countries. Do we need a different approach?
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2Technical Directorate for Eval of Plant Varieties and Plant Protect. Products, Madrid, Spain
3Directorate for Evaluation of Plant Protection Products, Oeiras, Portugal

The most recent scientific regulatory guidance document to conduct risk assessments for birds and mammals for plant protection products, issued by EFSA (2009), designates a tiered assessment framework.

In an active substance, and its associated product and use, fails the earlier stages (screening step and tier 1) of the tiered approach, it is possible to further refine the exposure evaluation level by using crop specific 'focal species' present at BBCH stages at the moment of the application. For birds and mammals a 'focal species' is a real species that is present in the crop when the pesticide is being used and is considered to be representative of all other species from the feeding guild that may occur in the crop at that time. Whereas several studies have been conducted in typical crops of central temperate Europe, less information is available for specific crops in Mediterranean countries.

New Article 110/70/2009 replacing Directive 91/414/EEC includes the concept of the zonal evaluation, and the subsequent mutual recognition of authorizations in order to facilitate harmonization of PPP in the EU and avoid duplication of work. From the experience gained from the majority of active substance evaluations, the risk assessment on birds and mammals requires higher tier assessments, which leads a considerable higher workload and expertise for risk assessors, regulators and notifiers.

Although FOCUS soundings face almost the same crops and weather conditions, there may be difference on perspectives as well as on protection goals. The basic characteristics of the Mediterranean agronomy and agro-environment (e.g. high diversity of environments, the intensive rotation in some crops), may facilitate/refine further the risk assessment on birds and mammals.

Here we suggest objective criteria for selecting suitable focal species to improve the interpretation of the risk assessment in Mediterranean agricultural landscapes and provide quality indications to identify protection goals and risk management decisions for Mediterranean countries in order to facilitate the development of a registration work-sharing programme.

WE 060
Focal species and their ecological behaviour in pesticide risk assessment in the Netherlands
J. Wassenberg
J . Wassenberg

The ecological behaviour of bird and mammal species is regularly used in refined risk assessments of pesticides in Europe. Also in the Netherlands applicants often choose this route to address risks indicated in the first tier. There is as yet no harmonisation between EU countries on the choice of focal species per crop, not on the required conservatism concerning the PT and PD values.

To aid the discussion in the session on focal species of birds and mammals and their ecological behaviour for refined risk assessments of plant protection products in Europe, this poster will provide a review of focal species with PD and PT used in risk assessments of pesticides in the Netherlands in the past years and considerations on the assumptions behind these choices.
WE 061
Experiences with the application of the EFSA Birds and Mammals guidance
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The EFSA Bird and Mammal guidance document was first published in 2009 to supersede SANCO/4145/2000. The various assessments within the guidance have been expanded and updated based on more recent information/data. Since it’s use, in both new assessments and updating previous applications using the old SANCO guidance, it has become apparent that the impact of the changes to the outcome and interpretation of the risk assessments. Focusing on the most commonly used assessment of dietary risk, the differences between the two guidance documents are substantial, although still maintaining the tiered approach.

Comparison of the step approach and selection of crop group/species scenarios provides a clear understanding as to the factors driving the assessment and how potential differences can occur in the assessment outcome. The updating of applications to the EFSA bird and mammal assessment reveal the impact of the new guidance can generally provide more favourable conclusions in demonstrating an acceptable risk. However, there are also examples that show little difference in the overall outcome and even instances where the EFSA guidance highlights an unacceptable risk which was not apparent under the old SANCO guidance. The EFSA guidance utilises the ‘Generic Focal Species’ and ‘season’ based on more representative data than the old SANCO ‘Indicator Species’ and therefore failure at this stage can require greater consideration for refinement than under the old SANCO guidance. This makes the selection of the ‘Focal Species’ in refinement particularly critical and as further data becomes available, PD and PT factors need particularly consideration as they play a more influential role in demonstrating safe use.

WE 062
Ecological context of long-term avian risk assessments: UK spring seed treatment example
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Long term avian risk assessments for seed treatments have a relatively high failure rate. Refined risk assessments may include focal species and associated dietary parameters (e.g. literature or field data for PD, PT). Compound specific seed residue decline data may also be used. Despite such refinements, however, the risk assessment may still indicate potential for concern.

One way forward is to place the risk assessment into an ecological context. For spring and summer applications especially, concern centres around exposure of birds during the breeding period (EFSA, 2009). Risk assessments also tend to be conducted on a one-field basis, equivalent to exposure of birds across the agricultural landscape. For relatively minor crops, however, this may not be the case.

Here, we show how information on the timing of breeding activity and timing of application (chronological aspect) may be combined with information on cropping density in selected focal regions (spatial aspect) to estimate the proportion of breeding activity in a focal species to which the long term toxicity/exposure ratio (TERLT) would apply. For a spring barley seed treatment in the UK, we show that the TERLT would apply to approximately <1% of breeding activity in the Skylark (for which the refined dietary TERLT was <1). Also, using breeding data, we propose an alternative focal species for which breeding would coincide to a greater extent with the exposure window (i.e. worst-case timing).

Overall, therefore, assessing ecological context allows for added realism in risk assessments.

WE 063
Assessing avoidance in a series of modified laboratory and cage tests with birds
M. Fountaakis
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According to EU procedures for the registration of plant protection products, potential risk for the avian community is identified, apart toxicity, on the basis also of a theoretical risk assessment scenario. In this study, we report on the three possible designs for the experimental avoidance testing in the field, compare these designs and discuss their potential. In this study, we attempt to present a sequence of methods that takes into account the avoidance behavior in the evaluation of slightly modified 5 days “no-choice test”, 3 days “two-choice test” and a proposed modified 3 days semi field (“cage test”) method with birds in order to evaluate the food avoidance behavior.

In this study, we report on the three possible designs for the experimental avoidance testing in the field, compare these designs and discuss their potential. This report describes three basic test designs to determine the avoidance behavior of birds and their contribution to exposure to pesticides. There are different options to quantify the degree of avoidance. The determination of avoidance of treated food is possible either by a “no-choice test”, in which only treated food is offered ad libitum during the exposure period or by a “two-choice test”, in which both treated and untreated food are offered to the birds at the same time.

In our study, we present a sequence of methods that takes into account the avoidance behavior in the evaluation of slightly modified 5 days “no-choice test”, 3 days “two-choice test” and a proposed modified 3 days semi field (“cage test”) method with birds in order to evaluate the food avoidance behavior.

This method is based on the investegation of the question whether there are consistent differences between the three possible designs but also provides information necessary to understand the intrinsic parameters, possible learning abilities but also other factors that may contribute to the overall avoidance response to a pesticide. It also provides evidence to decide whether risk is substantially reduced or not by avoidance in the field.

A case study is presented and a refine risk assessment for plant protection products is discussed further under the light of the most recent scientific regulatory guidance document to conduct risk assessments for birds and mammals for plant protection products issued by EFSA [EFSA Journal 2009, 7(12): 1438]. The use of avoidance testing in the context of current European Regulations for plant protection products will be discussed.

WE 064
Risk regulation for farmland bird and mammal species by accounting for indirect effects of pesticides
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The current state of scientific knowledge indicates that indirect effects of plant protection products (PPPs) can significantly contribute to the risk for non-target species in agricultural landscapes. The lack of understanding of the occurrence of environmental effects on the environment including biodiversity and to attain a sustainable use of pesticides it is obvious that indirect effects cannot be ignored in the risk regulation of PPPs. One of the essential prerequisites for the development of efficient risk management strategies for indirect effects is the identification of the species concerned, especially their ecological requirements, with focus on food availability and potential interferences with the timing of PPP applications.

We conduct a comprehensive literature review on effects of PPPs on a range of bird and mammal species that occur in German arable landscapes, with special focus on indirect effects on biodiversity. From this data we develop a sensitivity index to assess the potential risk a species is facing from effects of PPPs. We then use this index to calculate vulnerability indices that compare and evaluate the influence of different crop types or seasonal and regional variations in PPP applications. The vulnerability index, based on species-specific features, allows for making potential and actual hazards of pesticides visible. Results from this study provide the definition of focal species in German agricultural landscapes with a specific focus on indirect effects and highlight the necessity of comprehensive risk assessment strategies.

Indirect effects of PPPs threaten populations of wildlife species in arable landscapes. The identification of umbrella species is found to be an essential prerequisite to derive effective measures which can compensate or minimize the risk from indirect effects for a broad suite of affected species. We here represent a tool to further enhance existing risk management strategies in order to achieve the overall aim of a sustainable use of PPPs.

RA10P - Global Mercury: Bridging science and policy

WE 066
Human risk through consumption of European sea bass contaminated by mercury: (re)definition of edible parts
L. Mistro, M. Pacheco, A.C. Duarte, M.E. Pereira
CESAM, University of Aveiro, Aveiro, Portugal

Juvenile specimens of Dicentrarchus labrax (European sea bass) were collected at three different sites along an anthropogenic mercury (Hg) contamination gradient during the exposure period or by a “two-choice test”, in which both treated and untreated food are offered to the birds at the same time.

Experiences with the application of the EFSA Birds and Mammals guidance to conduct risk assessments for birds and mammals for plant protection products issued by EFSA [EFSA Journal 2009, 7(12):1438]. The use of avoidance testing in the context of current European Regulations for plant protection products will be discussed.

WE 067
Hg bioaccumulation in shoots of the macrophyte Elodea nuttallii in the field and in the microcosm
C. Georas, A. Garcia Bravo
Forei Institute, Versoix, Switzerland

Here, we show how information on the timing of breeding activity and timing of application (chronological aspect) may be combined with information on cropping density in selected focal regions (spatial aspect) to estimate the proportion of breeding activity in a focal species to which the long term toxicity/exposure ratio (TERLT) would apply. For a spring barley seed treatment in the UK, we show that the TERLT would apply to approximately <1% of breeding activity in the Skylark (for which the refined dietary TERLT was <1). Also, using breeding data, we propose an alternative focal species for which breeding would coincide to a greater extent with the exposure window (i.e. worst-case timing).

Overall, therefore, assessing ecological context allows for added realism in risk assessments.

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RA10P - Global Mercury: Bridging science and policy
bioaccumulation was measured in several macrophytes collected in Babyen reservoirot. Elodea nuttallii showed high Hg accumulation including methyl-Hg in shoots and a broad distribution in the river. We therefore further studied this plant in the laboratory. Tolerance and accumulation of inorganic and organic Hg in E. nuttallii was concentration dependent. Transportal transport of Hg from shoots to roots was predominant. Hg concentrations were higher in roots>leaves>stems and in top-middle>bottom of shoots. In shoots, 63±11% and 45±17% of Hg were found in cytosol and cell walls respectively. Accumulation was highly reduced by cold, death and by competition with Ca+

2. Results and discussion

Broad distribution in the river. We therefore further studied this plant in the laboratory. Hg (as high as 3.9 ppm in sharks, prompting the first marine fish advisory, and 4 ppm in king mackerel, observed in the cell wall whereas uptake in the cytosol was linked to the metabolism probably through copper transporters. Elodea nuttallii does not seem to represent a major form of mercury from sediments to the field herbivores may be exposed to Hg through ingestion of plant parts. Our study showed that more knowledge concerning Hg accumulation mechanisms in plants will be valuable to understand Hg bio-geochemical cycles in the environment.

3. Conclusions

E. nuttallii showed a high accumulation and tolerance to Hg in the field and in the microcosm. Hg in shoots and roots was specifically from the water column. Passive diffusion was observed in the cell wall whereas uptake in the cytosol was linked to the metabolism probably through copper transporters. Elodea nuttallii was a common species in the coastal ecosystem. Our study showed that more knowledge concerning Hg accumulation mechanisms in plants will be valuable to understand Hg bio-geochemical cycles in the environment.
species from Florida Bay in the 2000s compared to 1990s. Hg levels in some individuals of various taxa exceed risk benchmarks for fish and wildlife. A recent study indicates two primary drivers: very high atmospheric Hg deposition, reflecting a combination of high rainfall (30+ yr. mean of 131 cm) with high Hg (2009 ann. vol.-wt. mean =12.6 mg/nm) and sulfite contamination of Everglades’ surface waters (up to 60 mg/l). When these drivers are overlain on the low elevation (mean elevation of 0.5 m above mean sea level, slightly below the Everglades landscape) and low relief or water level (from rains or tides) produce dramatic variations in methylmercury production across vast wetland expanses (>9054 km²). Connectivity between ecosystems is a current research focus. The current management strategy includes: 1) advisories to protect human health (“no consumption” for 17 fish species across the Everglades and “limited consumption” for >60 species in coastal waters); 2) developing a statewide total maximum daily load (TMDL) for the freshwater ecosystem; 3) assessing the need to manage sulfur in the Everglades; and 4) developing a Gulf of Mexico TMDL.

WE 073
A preliminary assessment of Hg contamination in Lake Chapala, Mexico
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Texas A&M University, College station, United States of America
CIDIR, IPN, Juquiapan michoacan, México

The Lerma-Chapala Basin concentrates about 10% of Mexico’s human population. Lake Chapala is the largest tropical lake in Mexico and the ultimate receptor of a great variety of wastes that are discharged through the basin to the Rio Lerma. In addition to being a major water source for the city of Guadalajara, Chapala Lake represents a major fishery and recreation resource for various communities surrounding the Lake, as well as for tourists from many parts of the country. Chapala Lake is one of the largest wintering areas for American white pelicans (Pelecanus erythrorhynchos), a species of special concern in the United States. We are currently conducting an in-depth analysis in 2013 of the impacts on Hg, among other metals, with a pilot study conducted in 2012. Our working hypothesis is that the lake could become a natural sink for Hg, as similar to what has been observed in other parts of the world (e.g., Lake Erie, New York). Our working hypothesis is that the lake could become an important source of Hg, as similar to what has been observed in other parts of the world (e.g., Lake Erie, New York).

WE 074
Effect of synthetic organic compounds on the toxicity and uptake of mercury by a unicellular green alga
S. Le Faucheur, C.E. Portilla Castillo, V. Slavekova
University of Geneva, Versoix, Switzerland

Mercury is a widespread contaminant of highly concern for governments due to its biomagnification (as methylmercury - MeHg) along the food chain and its consequent impacts on top consumers, including humans. Phytoplankton is the first entry of Hg into the trophic chain and the major step in the biomagnification process. In the knowledge of parameters controlling mercury toxicity and uptake by algae is thus proverbial to establish relevant water quality criteria.

In natural waters, algae are exposed to numerous inorganic and organic contaminants, whose mixture can be potentially more toxic than the exposure to one contaminant at a time. The aim of this study is to study the compound, on MeHg toxicity and uptake by unicellular green algae. Our working hypothesis is that PFOS will increase algal membrane permeability, leading to more passive diffusion of MeHg (uptake) through the algal membrane and thus more toxicity for the algae.

Up-take of MeHg by C. reinhardii is induced to increased concentrations of MeHg and PFOS alone, in combination, and examined for its growth and the modes of action of the studied contaminants using a flow cytometer. Modification of membrane permeability and production of reactive oxygen species are both assessed using proper bioimaging dyes.

WE 075
Effect of synthetic organic compounds on the toxicity and uptake of mercury by a unicellular green alga
Corina Mundaca, Guadalupe Diaz, Jorge Nriagu
CSIC, Barcelona, Spain

Institute of Environmental Diagnosis and Water Studies, CNMC, Barcelona, Spain

We determined levels of mercury and methyl mercury (MeHg) in yellow-legged gull (Larus michaelsi) eggs from 8 locations in the Iberian Peninsula. These areas represent the most important gull colonies of the area and they are all declared as Special Protection Area for birds. We also examined eggs from Audouin's gull (Larus audouinii), one of the most endangered gulls in the world, in three different areas of the Ebro Delta. In the main aims of this study were to evaluate i) the spatial distribution, according to site dependent anthropogenic pressure, of Hg and MeHg using Larus michaelsi eggs and ii) differences in metal levels in Larus michaelsi and the protected species Larus audouinii from the Ebro delta, the world's largest Audouin breeding colony.

Hg and MeHg concentrations in the yellow-legged gulls ranged between 0.46 - 1.36 mg/Kg dry weight and 0.38-0.69 mg/Kg dry weight respectively. Significant differences for both Hg and MeHg were only found between eggs from Chafarinas and the Ebro Delta (a hot spot for Hg contamination). In yellow-legged gulls, MeHg represented between 23-57% of the total mercury, for the eggs of other species. At all sites, the highest Hg and MeHg concentrations were obtained from samples collected at the breeding grounds, with no significant variation between years in any of the areas.

In the areas of the Ebro Delta, Audouin's gull’s eggs showed significantly higher concentrations of Hg (4.64-5.37 mg/kg dry weight) and MeHg (1.5-3.0 mg/kg dry weight) than yellow-legged gull eggs. The correlation between total mercury and methyl mercury concentrations in fish to antioxidant responses in different seasonal periods.

The different response patterns, according to the year period, also revealed that the environmental conditions influenced the total Hg threshold able to induce or inhibit the antioxidant defences, highlighting the importance of evaluating the fish antioxidant responses between year periods showed that the warm period was the most critical with brain’s responses evidencing both adaptive mechanisms and signs of tissue degradation. The antioxidant responses in fish from the Ebro Delta are amongst the highest values reported for seabirds. However, a high Hg concentration in Audouin’s has not been clearly correlated with hatching failures.

WE 076
Distribution of mercury in water, sediment and fish from the Volta Lake and its major tributaries
E.E. Kwaansa-Ansah1, A.A. Adimado1, J.H. Ebrahim1, D. Nam1, J.O. Nriagu1
Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Catholic University College, Fipre-sunyani, Ghana

School of Public Health, University of Michigan, Ann arbor, United States of America

Concentrations of total mercury and methyl mercury were determined in fish and sediment from the waters of the Volta Lake and its main tributaries to understand their distribution in the ecosystem. Total mercury concentrations were determined in fish from populations ranging from 1.77 to 319.48 (mean: 68.44) µg/kg wet wt. and accounted for the total mercury in the muscles of the fish. Methyl mercury concentrations in fish were directly proportional to total mercury concentrations with a correlation coefficient of r = 0.98. The relationship of total mercury and methyl mercury concentrations in fish to toxic and technology correspondences in the brain of wild European sea bass (Dicentrarchus labrax) during two different year periods (warm and cold). The selected parameters were total glutathione (GSH) content as non-enzymatic antioxidant, catalase (CAT), glutathione peroxidase (GPOs), glutathione reductase (GR) and glutathione S-transferase (GST) activities as antioxidant enzymes, and lipid peroxidation as a biomarker for tissue damage. This revealed a significant response of different environmental Hg loads in the Rio de Janeiro and Lagos (Portugal) a reference area, and two sites at the Laranjo area (a moderately and a highly contaminated site). D. labrax demonstrated a complex profile marked by toxicity signs (as CAT inhibition) and adaptability (as GR induction), indicating different toxicity thresholds, depending on the measured parameter. The comparison of the antioxidant responses between year periods showed that the warm period was the most critical with fish’s responses evidencing both adaptive mechanisms and signs of tissue damage. No alteration in the oxidative status of antioxidant enzymes were observed, although some signs of toxicity could be evidenced by the depletion of specific antioxidant responses, no lipid peroxidation occurred in any of the studied periods. The different response patterns, according to the year period, also revealed that the environmental conditions influenced the total Hg threshold able to induce or inhibit the antioxidant defences, highlighting the importance of evaluating the fish antioxidant responses in different seasonal periods.

WE 077
Fish brain as a critical target of mercury exposure: the importance of seasonal factors
L. Vieiro, M.E. Pereira, A.C. Duarte, M. Pacheco
CESAM, University of Aveiro, Aveiro, Portugal

Mercury’s (Hg) neurotoxicity ability has been strongly recognized in both humans and wildlife, being brain one of the primary targets for its toxic effects. Hg neurotoxicity is widely studied in mammals but not so frequently in fish. Nevertheless, some brain disturbances have been addressed, namely brain sensitivity to Hg induced oxidative stress. An increase in brain levels of Hg in populations at risk was studied in populations of freshwater fishes in the brain of wild European sea bass (Dicentrarchus labrax) during two different periods (warm and cold). The selected parameters were total glutathione (GSH) content as non-enzymatic antioxidant, catalase (CAT), glutathione peroxidase (GPOs), glutathione reductase (GR) and glutathione S-transferase (GST) activities as antioxidant enzymes, and lipid peroxidation as a biomarker for tissue damage. This revealed a significant response of different environmental Hg loads in the Rio de Janeiro and Lagos (Portugal) a reference area, and two sites at the Laranjo area (a moderately and a highly contaminated site). D. labrax demonstrated a complex profile marked by toxicity signs (as CAT inhibition) and adaptability (as GR induction), indicating different toxicity thresholds, depending on the measured parameter. The comparison of the antioxidant responses between year periods showed that the warm period was the most critical with fish’s responses evidencing both adaptive mechanisms and signs of tissue damage. No alteration in the oxidative status of antioxidant enzymes were observed, although some signs of toxicity could be evidenced by the depletion of specific antioxidant responses, no lipid peroxidation occurred in any of the studied periods. The different response patterns, according to the year period, also revealed that the environmental conditions influenced the total mercury’s threshold able to induce or inhibit the antioxidant defenses, highlighting the importance of evaluating the fish antioxidant responses in different seasonal periods.
Mercury (Hg) is one of the most hazardous heavy metals usually found in aquatic environments. Methyl mercury (MeHg), one of the toxic organic forms, can bioaccumulate and biomagnify along the trophic chain. Inorganic mercury ([Hg] and MeHg have been found in the sediments and settling particles in Lake Geneva (Switzerland). Vidy Bay is the southern embayment of Lake Geneva, and receives wastewater discharges from a Sewage Treatment Plant (STP), located in the city of Lausanne. These discharges also make this area rich in bacteria. The highest concentrations of Total Mercury (THg) and MeHg in sediments are located around the STP (1.32 mg/kg and 5.2 µg/kg respectively) as compared to concentrations further away from the source (0.17 mg/kg and 0.36 µg/kg) and also prior to the STP implementation (0.04 mg/kg for THg). With respect to settling particles, MeHg concentrations on settling particles varies greatly in the water column between the upper (75m below the surface) and lower sections of the bay (1 to 16 µg/kg), without any apparent seasonal patterns.

Methylation is shown to be carried out by sulfate and iron reducing bacteria whose metabolism transforms HgII into MeHg in lake sediments (Garcia-Bravo 2010). Moreover, settling particles from the water column were filtered and analysed to rule out any input of MeHg from the water column itself. It is believed that due to the environment created within sediment traps, the growth of bacteria can be promoted, entailing methylation processes more easily than in raw sediments, as implied from a decreasing oxygen content observed down the sediment trap tube. In light of these results, MeHg couldn’t be used as a tracer to predict the transport of particle-bound pollutants and particle resuspension.

Critical Hg concentrations in atmospheric deposition and fish for a sensitive lake: Lake Mergozzo (northern Italy)

L. Guzzì, D.A.L. Vignati, A. Novo, P. Guizzoni

The applicability of silver discs to trap Hg in precipitation was confirmed by the use of Pure silver 0.8 µm mesh filter layers placed holding natural litter homogeneously spiked with 202Hg. Five replicate EMs in two clusters were set-up at the field site to represent a clearing and an under canopy plot respectively. For each sampling period and EM, a 15 g sample of freshly prepared litter spiked with 150 ng 202Hg was applied. Subsequently, the exposed litter and silver discs were replaced and brought back to laboratory.

Settling particles from the water column were filtered and analysed to rule out any input matter released from the STP. Sediment traps, exposed to an antibiotic mixture tested to kill the bacteria living on the collected particles, showed a significant difference in MeHg concentrations as compared to sediment traps without antibiotic exposure, over the same periods, in laboratory tests.

The deep, large subalpine lakes (DSL), located south of the Alps between Italy and Switzerland, are regionally important economic and environmental resources. DSL share several common morphological features (e.g., narrow and elongated shapes, steep sides, considerable depths, and most often north-south orientation), although the lithological and petrographical composition of their watersheds differs markedly because of the geological complexity of the Alpine region. Most of the DSL experienced eutrophication during the 20 century and their present trophic status varies from oligotrophy (e.g., Lake Maggiore, thanks to recovery measures) to eutrophy (e.g., Lake Lugano). To assess how different trophic states influence the Hg cycle, two lakes covering the last 100 to 700 years (depending on core length and sedimentation rates) were selected at locations of DSL. The deepest sections of each core were usually free from human influences (pre-1850) and show that background Hg levels (0.05-0.1 mg kg⁻¹) are similar across the DSL despite differences in catchment composition. Mercury content in the top-layers of the cores remains above background levels in all DSL and moderate to strong anthropogenic impact is observed not only in Lake Maggiore (for which a well-documented history of Hg pollution exists), but also in lakes Orta and Como. Analysis of the complete Hg profiles is being undertaken and will elucidate how Hg deposition in DSL responds to changes in trophic status and regional climate parameters.

Mobility and reactivity of mercury deposition to the litter layer of a Chinese subtropical forest: results from a study including addition of a stable Hg isotope to novel in-situ exchange devices

H.Z. Zhang, F.E.N.G. Ximbin, S.O.M.M.A Jonas, N. Luca

Mercury (Hg) is one of the most hazardous heavy metals usually found in aquatic environments. Methyl mercury (MeHg), one of the toxic organic forms, can bioaccumulate and biomagnify along the trophic chain. Inorganic mercury ([Hg] and MeHg) have been found in the sediments and settling particles in Lake Geneva (Switzerland). Vidy Bay is the southern embayment of Lake Geneva, and receives wastewater discharges from a Sewage Treatment Plant (STP), located in the city of Lausanne. These discharges also make this area rich in bacteria. The highest concentrations of Total Mercury (THg) and MeHg in sediments are located around the STP (1.32 mg/kg and 5.2 µg/kg respectively) as compared to concentrations further away from the source (0.17 mg/kg and 0.36 µg/kg) and also prior to the STP implementation (0.04 mg/kg for THg). With respect to settling particles, MeHg concentrations on settling particles varied greatly in the water column between the upper (75 m below the surface) and lower sections of the bay (1 to 16 µg/kg), without any apparent seasonal patterns.

Methylation is shown to be carried out by sulfate and iron reducing bacteria whose metabolism transforms HgII into MeHg in lake sediments (Garcia-Bravo 2010). Moreover, settling particles from the water column were filtered and analysed to rule out any input matter released from the STP. Sediment traps, exposed to an antibiotic mixture tested to kill the bacteria living on the collected particles, showed a significant difference in MeHg concentrations as compared to sediment traps without antibiotic exposure, over the same periods, in laboratory tests.
The general public usually recognizes the existence of benefits and risks connected with fish consumption, but often has very poor knowledge of the specific benefits, of the risks of the contaminants of concern, and of the ‘risky’ types of fish products. In the case of mercury, scientific knowledge increasingly confirms that, in the absence of local pollution factors, very high Hg content is limited to a few top-predator species. Given this situation, the mass media have the important task to correctly pass on the available scientific knowledge to the public for a correct choice (in terms of species and quantity) of the fish products to be preferred. To evaluate to what extent such ‘transmission’ is taking place, we assessed how the benefits vs. risk of fish consumption are represented in the two main Italian broadsheets. In parallel, we collected hair samples and information on dietary habits from volunteers residing in various areas of northern Italy. Finally, we compared Hg content in some fish products available on the Italian market from both Italy and Spain with the results with the existence of relevant Hg species. Our socio-scientific approach shows that further work is needed to translate scientific evidence on Hg-related health risks into an appropriate communication framework.

**WE 084**
The use of isotopic tracers to study transformation potentials and subcellular localization of Hg species by a sulfate reducing bacteria M. Monperrus, Z. Pedero, B. Maite, F. Pannier, S. Mounicou, D. Amouroux, R. Guyoneaud

**WE 085**

**WE 086**
Simultaneous environmental exposure of biocides - potential underestimation in the environmental risk assessment K. Michaelis1, M. Kasper, K. Ziegler, I. Beer, I. Nohr, R. Groβ, A. Hermann3, S. Gartiser1

**WE 087**
Risk mitigation measures for the authorisation of disinfectants - developing guidance for harmonisation S. Gartiser1, J.L. Jager2, M.K. Michaelis1, W.S. Wiek1, E.E. Petersohn2

**WE 088**
Toxification of polyDADMAC, KC1 and alumina to Corbicula fluminea and non-target aquatic species J.M.F. Gomes1, J.C. Rosa2, P.M. Saraiva2, F. J.M. Gonçalves1, J.L Pereira1, R.J. Costa3

**RA12P - Health and environmental risk assessment of pesticides and biocidal products**
freshwater-dependent industries due to their biofouling activity. Depending on the way industries interact with freshwater ecosystems, the chemicals currently available for biofouling control may have high toxicity to non-target species, and hence improved methods with enhanced environmental acceptance are required. This rationale triggered the development of an alternative to the traditional biocides currently used in waterworks. This alternative was prepared by coupling polyDADMAC and D-glucan (a mannose-rich biopolymer) to form polyDADMAC-decorated D-glucan (polyDADMAC-D-Glu).

The aim of the present research was to examine the extent of release of toxic substances from wood materials treated with wood preservatives and to explore efficiency of paint treatment in reduction of leaching of toxic substances. Concrete tests were conducted with untreated and naturally weathered wood materials treated with three different wood preservatives were tested - D.D. COP, D.D. Waterguard and D.D. EcoGuard. The research was carried out in collaboration with ESF projects 2009/0226/1DP/1.1.1.2.0/09/APIA/VIAA/005 and 2009/0144/1DP/1.1.2.1.2/09/APIA/VIAA/005.

WE 091
Ecotoxicity of leachates from wood materials treated with wood preservatives
1. Putna, J. Simonavicsa, K. Valters, G. Bzabamirs, S. Purriva, M. Balode
2. Latvian University of Agriculture, Riga, Latvia
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Wood materials are often used for outdoor constructions. It is expected that with growing demand for natural and renewable materials their usage will even increase in the future. In order to protect the wooden outdoor construction biocides (wood preservatives) are applied, and if they are released to the environment with run-off waters, this could be considered as a source of environmental pollution with highly toxic substances. Due to the high toxicity, some of the historic wooden preservatives are banned or heavily restricted e.g. creosote, pentachlorophenol. There are several possibilities to reduce the environmental pollution coming from outdoor wooden constructions, e.g., by using modified preservatives with less toxic chemicals as components where possible, by reducing contact with water, or by achieving stronger binding of the toxic substances to the wood material. The precondition for all these actions is proper information about the presence and concentrations of toxic substances in the composition of wood preservatives.

The purpose of the present research was to detect the extent of releases of toxic substances from wood materials treated with wood preservatives and to explore efficiency of paint treatment in reduction of leaching of toxic substances. Leachates obtained from painted and unpainted wood materials treated with three different wood preservatives were tested - D.D. Cop, D.D. Waterguard and D.D. EcoGuard. The research was carried out in collaboration with ESF projects 2009/0226/1DP/1.1.1.2.0/09/APIA/VIAA/005 and 2009/0144/1DP/1.1.2.1.2/09/APIA/VIAA/005.

WE 092
Pesticide levels in vegetables and related health risk assessment in Central Amazon - Brazil
S. Campos, V. Wachman
UNIVALI, SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting

Food consumption is an important route of human exposure to pesticides. In the Brazilian Amazon, intensive and incorrect use of pesticides in floodplain areas, where fresh vegetables are produced for regional urban markets is a common practice. As a consequence of that, it is possible that pesticide residues on vegetables are above of the maximum residue limits (MRL) and consequently could result in health risks for consumers. The objective of this study was to determine pesticide residues in vegetable samples from the Central Amazon region to assess the level of exposure and to evaluate potential health risks. In this study, 300 samples of vegetables were collected in 10 different localities in the Central Amazon region between June and September 2009. Pesticide residues in vegetables were analyzed by headspace solid phase microextraction (HS-SPME) and gas chromatography with electron capture detection (GC-ECD).

The detection of pesticides in vegetables is important to ensure the safety of the consumer. Therefore, the assessment of pesticide residues in vegetables is necessary to ensure compliance with the maximum residue limits (MRL) established by the Brazilian Federal Government. The present study was performed in the co-operation among ESF projects 2009/0226/1DP/1.1.1.2.0/09/APIA/VIAA/005 and 2009/0144/1DP/1.1.2.1.2/09/APIA/VIAA/005.
Over the 42-d experimental period the analytically determined time-weighted average concentrations of azoxystrobin ranged from 93.5 to 99.3 % of intended time-weighted average concentrations. The study showed that zooplankton and especially copepods and Daphnia group longispina were the most sensitive groups for a chronic exposure of azoxystrobin.

Mammalian and fish studies did not show significant treatment-related effects as also was the case for macroinvertebrates, phytoplankton, decomposition of organic matter and water quality parameters. Based on the responses of the copepod population a consistent NOEC was calculated at the 1 µgL−1 treatment level. At the zooplankton community level at concentrations up to and including 10 µg a.l.L−1 no consistent treatment-related effects were found. Most treatment-related effects observed were not followed by reductions in the exposure regime which were maintained until the end of the experiment. The chronic threshold value was approximately a factor of 10 lower than was found for a single application of azoxystrobin in an outdoor microcosm study (CTGB, www.ctgb.agro.nl). The safe threshold values for chronic exposures at the population and community level suggest that the first-tier value for chronic exposures (44 µgL−1 NOEC for Daphnia magna)/10) = 4.4 µgL−1 is not protective for chronic effects at the population and community level.

**WE 094**

**Highest tier EPAT analysis in aquatic risk assessment of crop protection products**

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For the assessment conducted during the EU review process of plant protection products (PPP), the concentration of active substances in water bodies adjacent to a single field is calculated using the surface water model FOCUS SWASH. For higher tier assessment, micro- and mesocosm studies or probabilistic approaches (e.g. SSD - Species Sensitivity Distribution) can be used. On the exposure side most realistic PEC calculations by means of mitigation measures such as buffer zones or drift reducing nozzles or the use of less chronic endpoints are highly recommended. However, for the FOCUS D scenarios - in particular for D2 - it has been taken into account that winter cereals and winter oilseed rape - only limited mitigation measures are available as drainage is the main entry path. In such cases, an analysis of the exposure profile and water sediment may give valuable information for a successful estimation of risks to aquatic organisms. For this reason the extension of the EPAT approach to cover the exposure of investigated substances in aquatic surface water is necessary. The main focus was on specific compounds that enter the aquatic environment via drainage. By this, potential uses of EPAT as a higher tier refinement tool for acute and chronic risk assessment is being examined.

**WE 095**

**Integrative approach for the risk assessment of freshwater reservoirs influenced by intensive agricultural activities:** a case-study of Alqueva reservoir (South of Portugal)

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Freshwater reservoirs located in intensive agricultural areas are more vulnerable to chemical 'stressors', such as pesticides. So, the characterization of these water bodies for prevalent pesticides is extremely important, once most of these compounds are used in an indiscriminate way by farmers and induce toxic effects in species of aquatic ecosystems and their communities. The purpose of this study was to evaluate the risk of acute and chronic pesticides and its correlation with the toxicity values obtained in the Alqueva reservoir, an important source of water for irrigation and supply (South of Portugal).

Water and sediments samples from the Alqueva reservoir were analysed from the first to the last week of its lifetime (from the time of application until harvest). Residues in both whole fruits and marketing units of Cali, Colombia. Pesticide concentrations were measured periodically in passion fruit samples from the time of application until harvest and during final consumption, the amount of spray deposition on plant surface, uptake processes, dilution due to crop growth, metabolism in plant material, and loss due to food disposal decision-making tools in these domains. In such a context, a Life+ project, Control of noxious or vector mosquitoes: implementation of integrated management of the spread of introduced tropical Aedes albopictus in Europe, causing the first chikungunya epidemic in Italy, have led the national and European authorities to become aware of the critical need of toxic effects observed in each location of the Alqueva reservoir, we can establish a possible relation-ship between pesticide concentrations found and the toxic effects observed in the bioindicators used. Taking into consideration the pesticides concentrations found and the toxic effects observed in each location of the Alqueva reservoir, we can establish a possible relation-ship between this environmental risk factor and the observed stress in the bioindicators used.

**WE 096**

**Reducing fish use in bioaccumulation studies for plant protection products**

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Fish bioaccumulation studies assist in determining the potential for substances to bioaccumulate. This is used for persistence, Bioaccumulation and Toxicity (PET) and secondary poisoning assessments. International data requirements for plant protection products include triggers for determining bioconcentration factor (BCF) values, generally where bioconcentration might be expected, e.g. for substances with an octanol-water partition coefficient (logKow) > 3 and that are stable in water. Bioconcentration tests are laborious, expensive, and use large numbers of animals (a minimum of 108 per study). After thorough review of the literature and with the FOCUS test committee it was proposed that BCF testing (TG 305) would therefore be of value in improving efficiency, reducing costs and supporting animal welfare considerations. Currently the OECD Test Guideline for BCF testing (TG 305) is being revised to include the possibility of reducing the cost and number of fish used, when this can be done without compromising the BCF determination. One potential modification that would substantially reduce fish use is to use only one exposure concentration, instead of the two currently required. Analysis of 53 studies on plant protection product active substances demonstrates that BCF values for whole body, edible and non-edible tissues do not significantly differ between these low and high exposure concentrations. One exposure concentration could therefore be employed for BCF testing of many substances, reducing fish use by one third. We recommend this modification is included in the upcoming revisions of OECD TG 305.

**WE 097**

**Setting up of validated procedures for the monitoring of the non-intentional effects of the control methods on man and the environment**

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7Centre de Démoustication, Conseil Général de Martinique, Fort-de-France, France

8Control of noxious or vector mosquitoes (Diptera-Culicidae) is an activity for which the socio-economic and environmental stakes are high. In France, given the importance of the services concerned, this public health mission was entrusted to specialised public operators. Recent events, such as chikungunya epidemic in La Réunion, the introduction of tropical Aedes albopictus in Europe, causing the first chikungunya epidemic in Italy, have led the national and European authorities to become well aware of the increased need for the evaluation of the efficacy, and the health and environmental risks pertaining to the current control strategies and to have at their disposal decision-making tools in these domains. In such a context, a Life+ project, Control of noxious or vector mosquitoes: implementation of integrated management for controlling populations and community level. Suggest that the first-tier value for chronic exposures (44 µgL−1 NOEC for Daphnia magna)/10) = 4.4 µgL−1 is not protective for chronic effects at the population and community level.

**WE 098**

**Pesticide residue dynamics in passion fruits grown in Colombia:** field trials, modelling, and human health risk assessment

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The use of synthetic pesticides from the cultivation of passion fruits and subsequent human health risks, we combine several methods: (i) experimental field studies including the determination of pesticide residues in passion fruits, (ii) dynamic plant uptake modeling, and (iii) concepts of human health risk assessment. Eight pesticides (azoxystrobin, difenacoum, terbutylazine, tebuconazole, deltamethrin, imidacloprid, thiamethoxam) were applied to passion fruits cultivated in the region of Cali, Colombia. Pesticide concentrations were measured periodically in passion fruit samples from the time of application until harvest. Residues in both whole fruits and fruit pulp only were analysed using gas- and liquid chromatography. Measured concentrations were compared with predicted residues calculated by using a dynamic and plant-specific pesticide uptake model, namely dynamiCROP. The model takes into account the time between pesticide application and crop harvest, the time between harvest and final consumption, the amount of spray deposition on plant surface, uptake processes, dilution due to crop growth, metabolism in plant material, and loss due to food residue. A pesticide residue dynamics model was developed which included the differentiation of pesticide residues in passion fruits, the development of the pesticide residues in the plant and the risk assessment. A pesticide residue dynamics model was developed which included the differentiation of pesticide residues in passion fruits, the development of the pesticide residues in the plant and the risk assessment.
Risk of pesticides in the air for organic farming

C.T. Turgut1, D. Ates1, L. Atanazi1, H. Ornek1
Adnan Menderes University, Aydin, Turkey
Namik Kemal University, Tekirdag, Turkey
Bernout Plant Protection Research Station, Izmir, Turkey
Over 750 million olive trees are cultivated worldwide, about 95% of those are in the Mediterranean region. Turkey accounted for over 9% of world production and is 4th biggest producer in the world. Over 24% of olive oil production comes from Province Aydin in Turkey. Pesticides are not or seldom used in olive trees, so Province Aydin is very suitable for organic olive oil production but passive accumulation of pesticides via short and long range transport of pesticide poses risk for olive trees and organic farming. The olive gardens in province Aydin were divided to 150 zone by help of GIS and the samples were collected in 2007 and 2008. The leaves were extracted and analysed by GC/MS/MS. The results showed that 19.33% of olive gardens was found as unsuitable for organic farming, although 80.67% of this area was suitable for organic farming. A map was created to ensure possible organic farming gardens in the province of Aydin.

MULTIPLE CONTAMINATIONS OF ORGANOPHOSPHORUS PESTICIDES AND HEAVY METALS AROUND THE RIVER REGION OF DAVE SMENTER, CHINA

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2Huangshi Institute of Technology, Huangshi, China
Numerous researches have been conducted on heavy metal contaminations around the river region of the Dave Smenter in China. This study is showing multiple contaminations of organophosphorus pesticides (OCPs) and heavy metals may occur in this region. Soil, vegetable, river water and sediment samples of the irrigation system around the Dave Smenter were collected for analyzing OCPs and heavy metals (As, Cd, Hg, Pb) in 2009. It was found that HCHs, DDTs, CHLs were the main OCPs' residual compounds. The concentrations of HCHs were higher than DDTs in most vegetable, soil and water samples. Composition analysis indicated that lindane (B-HCH) and aldrin-DDT may be input in this region recently. Relatively high concentrations of heavy metals were also found in the region, and their concentrations were generally higher in the Donggang River than in the Xigang River. Local residents may suffer from the health risk because of the multiple contaminations.

USING AN ECOSYSTEM SERVICES APPROACH TO ENABLE PRAGMATIC ENVIRONMENTAL DECISION-MAKING: A CASE STUDY ON CITRUS PRODUCTION AND INSECTICIDE USE

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The findings of this pioneering proof-of-concept study delivered a case study to inform discussions between scientists and policy makers in pesticide regulation where an ecosystem services approach can inform risk management decisions. An ecosystem services framework has been developed and is applied to a key insecticide for use in citrus growing in southern Spain. For more than 40 years, farmers in the region have relied upon an organophosphate insecticide as an important component of a pest control management strategy. The use of the pesticide limits the occurrence of blemishes on the surface of citrus fruit caused by red scale (Aonidiella aurantii), greatly effecting the marketability of the produce and the income earned by farmers at harvest time.

A spatial model was developed to evaluate the use of the insecticide in three citrus orchards in the Valencia Region. Specifically, the results of the evaluation were used to determine: (a) changes in ecosystem services in the absence of insecticide use; (b) the influence of other management actions on ecosystem services arising from the discontinued use of the insecticide; (c) the effect of the discontinued use of the insecticide; and (d) the possible temporal profile of changes in ecosystem services over a 50 year period such that strategies might be developed to minimise changes and enhance or preserve current ecosystem services and functions for future generations.

Conclusion of the scenarios against the baseline condition indicates the importance of the insecticide to commercial citrus production. In the event the use of the OP insecticide is discontinued, some ecosystem services and income are projected to decline. If use continues and potential impacts are offset with a mitigation action such as a vegetated conservation area, ecological services are projected to increase compared to baseline conditions, while preserving income and both amenity and carbon sequestration values.

Future directions for the project are defined.

ECOTOXICOLOGY AND MACROECOLOGY - TIME FOR INTEGRATION

M.A. Beketov, M. Liess
UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany
Despite considerable progress in ecotoxicology, it has become clear that this discipline cannot answer its central questions, such as, “What are the effects of toxicants on biodiversity?” and “How the ecosystem functions and services are affected by the toxicants?”. We argue that if such questions are to be answered, a paradigm shift is needed. The current bottom-up approach of ecotoxicology that implies the use of small-scale experiments to predict effects on the entire ecosystems and landscapes should be merged with a top-down macroecological approach that is directly focused on ecological effects at large spatial scales and consider ecological systems as integral entities. Analysis of the existing methods in ecotoxicology, ecology, and environmental chemistry shows that such integration is currently possible. Therefore, we conclude that to tackle the current pressing challenges, ecotoxicology has to progress using both the bottom-up and top-down approaches, similar to digging a tunnel from both ends at once.

Effects of landscape heterogeneity and sub-lethal pesticide exposure on dispersal of Asellus aquaticus

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The current bottom-up approach of ecotoxicology that implies the use of small-scale experiments to predict effects on the entire ecosystems and landscapes should be merged with a top-down macroecological approach that is directly focused on ecological effects at large spatial scales and consider ecological systems as integral entities. Analysis of the existing methods in ecotoxicology, ecology, and environmental chemistry shows that such integration is currently possible. Therefore, we conclude that to tackle the current pressing challenges, ecotoxicology has to progress using both the bottom-up and top-down approaches, similar to digging a tunnel from both ends at once.

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Does water network structure matter for the simulated recoveries of aquatic macroinvertebrate populations after pesticide stress?

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Little is known about the influence of spatial heterogeneity on the ecological risks of chemicals. Population model effects are interesting tools to be used in this question because they provide a cheap and fast, though simplified method of assessing large-scale effects. Individual-based population effect simulation models such as MASTEP are spatially explicit, but their spatial environment consists of a single linear circular connected waterbody. This study aims to evaluate if and how different levels of spatial complexity influence the impacts of hypothetical pesticides on populations of aquatic macroinvertebrates. The MASTEP population model for Asellus aquaticus was used and simulated with the spatial structure of a typical Dutch ditch network. For each of the included water bodies, concentration time curves were calculated. A local MASTEP model was connected to each of the sections of the water body network. Exposure concentrations were linked with effects by calculating daily mortalities of individuals.

RA14P - Landscape ecotoxicology and spatially explicit risk assessment

WE 104

ECOTOXICOLOGY AND MACROECOLOGY - TIME FOR INTEGRATION

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WE 105

Effects of landscape heterogeneity and sub-lethal pesticide exposure on dispersal of Asellus aquaticus

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WE 106

Does water network structure matter for the simulated recoveries of aquatic macroinvertebrate populations after pesticide stress?

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SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
following a stochastic death approach. Different scenarios were simulated in 10 replicates each, and the recovery times have been calculated as the number of days after the input of the pesticide, that population abundances in treated scenarios need to reach 95% of the level of the abundances in respective control simulations. Conclusions from a series of simulations show, that the influence of the water network complexity and of spatially explicit vs. non-explicit settings obviously depends on the toxicity of the concern the most difficult to understand, whereas the removal of water bodies varies. Quantifying the recovery time is therefore important for assessing the impact of pesticide inputs on water bodies.

In this study, we focus on modelling exposure using the skylark model. We have developed a spatially explicit, agent-based modelling framework SkyPig for modelling bird populations. Currently SkyPig incorporates two species: skylark and woodpigeon. The purpose of both models is risk assessment of pesticides in higher tiers. In this study we focus on illustrating the importance of spatial relationships at a landscape scale.

The importance of spatial relationships at a landscape scale has been established in a large number of recent studies. The potential confounding factors influencing these indices, when used as in situ markers of toxicity, are however poorly known. The aims of this study are (1) to quantify the relationships between these indices and trace metal (TMs) concentrations in small mammals. In autumn 2006, 859 wood mice (Apodemus sylvaticus) were trapped along a soil pollution gradient (3 pollution levels), in four different habitats to assess whether the same factors influence indices in different TM levels and to compare the indices in the different habitats.

The results of the study showed that the indices were not significantly correlated with the pollution level, indicating that the use of these indices as markers of toxicity is likely to be of limited value. However, the results also indicate that the indices are useful for identifying potential areas of risk.

We also suggest how this method can be effectively applied.

WE 109

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Spatial temporal exposure assessment in networks of small surface water bodies

In a landscape with sufficient biological control, the amount of pesticides used can be reduced. However, the use of pesticides itself has a direct negative impact on potential beneficial arthropods. This feedback loop is especially important in ecosystems where the removal of non-crop elements, the distance between them and the proportion of crop fields potentially benefiting from CBC can be maximized.

In this work we present a prediction model for the pesticide exposure in networks of small surface water bodies. The tool can be used to analyse pesticide exposure patterns and indicate water segments of potential concern. The tool is configurable to develop and simulate management strategies which avoid the pesticide pollution of water bodies.

WE 110


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Body condition and somatic indices related to trace elements accumulation in small mammals

Body condition is one of the most widely used ecologists as estimators of wild animal health status, because they are hypothesized to be representatives of individual health and fitness. The potential confounding factors influencing these indices, when used in situ markers of toxicity, are however poorly known. The aims of this study were therefore to establish the relationship between body condition and somatic indices, when used as in situ markers of toxicity, and to determine the potential confounding factors influencing these indices.

The body condition of the small mammals was assessed using the Body Condition Score (BCS) and the Fat Condition Score (FCS). The BCS is a subjective index of body condition that ranges from 1 (emaciated) to 5 (extremely fat). The FCS is a subjective index of fat condition that ranges from 1 (very thin) to 5 (extremely fat). The BCS and FCS were computed on a scale of 1 to 5.

High levels of TMs were found in small mammals, indicating both an important transfer in food webs and a potential risk for these organisms. Relationships between indices and individual variables showed that the SMI varied according to both age and gender (p<0.05, partial R² of 0.08 and 0.22%, respectively). SLM and SLD were influenced only by age (p<0.05, partial R² of 0.06 and 0.23, respectively). Land use, which has only been scarcely studied previously in ecotoxicology, significantly influenced the indices, individuals living in woodlands and agricultural lands exhibiting significantly higher SMI and lower SLM and SLD compared to those living in shrublands. After taking into account those confounding factors (relevant individual variables and landscape), we failed to detect any influence of TMs on body condition and somatic indices. This indicates that the indices are not sensitive to these markers and need to be reinterpreted if not used with caution. Further studies are still needed to better identify and quantify the role of ecological factors such as habitat or stresses (parasites, predation, etc.) on these indices, and to determine how body condition and somatic indices react to ecological change in ecosystems.

WE 111

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Exposure and ecological risk assessment of herbicides in Japan: risk calculation approaches focusing on space- and time varying herbicide exposure

Exposure to herbicides, a GBG-based multimedia modeling system (Grid-Catchment Integrated Environmental Modeling System, G-CIEMS) has been developed for predicting spatiotemporal dynamics of chemical concentrations in Japanese rivers. For effect assessment, Dynamic Energy Budget toxicity (DEBtox) models have been developed as tools that connect temporal change in exposure concentrations with effects on organisms. In this study, we developed an
We present options for risk mitigation measures, which consider Swiss agricultural schemes and land-use. The planned procedure involves four main steps:

- Improvements of data availability at National level, would allow the results of this work to be extended to the entire Italian agricultural landscape.
- The buffer zones needed when using conventional nozzles are greater than predicted Environmental Concentrations are derived, and compared with data on toxicity of active substances to aquatic organisms. In the UK, chlorpyrifos is a key active substance for pest control in fruit and arable crops. In some cases there are no alternative products.
- Predicted Environmental Concentrations are derived, and compared with data on toxicity of active substances to aquatic organisms. In the UK, chlorpyrifos is a key active substance for pest control in fruit and arable crops. In some cases there are no alternative products.
- The buffer zones needed when using conventional nozzles are greater than those factors and river water quality. We examined the period from 1997 to 2010 during which Chongqing urbanized significantly. In 1997 Chongqing, which is located in the southwestern region of China, was designated as the fourth Municipality administered directly by the central government besides Beijing, Shanghai and Shenzhen. It is the largest municipality in China in terms of area and population, and most of the Three Gorges Reservoir lies within it. Since 1997 the population of Chongqing increased from 2.8 million to 32.2 million and the gross domestic product increased from USD 540 million to USD 11200 million.

We predicted the potential exposure of small streams to insecticides (run-off potential - RP) and the resulting ecological risk (ER) for freshwater fauna on the European scale using a spatially explicit model. Then, the service provided by the landscape (reduction in ER) was calculated by comparing the ER of a landscape lacking conservation sources with that of the actual landscape.

The authorization process of plant protection products (PPPs) provides a risk assessment of surface water contamination, which has the main purpose of addressing the impact of PPPs on surface water quality and on aquatic ecosystems. If the risk assessment performed according the supported GAP show an unacceptable risk for aquatic systems, mitigation measures have to be applied to reduce the concentration of PPPs which can reach water bodies and, affect aquatic organisms.

Due to the absence of data at national level for Italian landscape and considering the increased importance of mitigation measures in PPPs, as also underlined by the Sustentability Use Directive to select the proper mitigation measures that better fit the environmental and agricultural characteristics of Italy.

The aim of this study is to evaluate the applicability of mitigation measures in the Lombardy region, combining the use of GIS and statistical approaches (multivariate analysis) to identify critical combination of mitigation buffer for water bodies versus the regional arable land. These multivariate analysis are based on a set of parameters, such as elevation, geo-pedological soil conditions, slope, distance between field and water bodies, which are considered representative of the Regional arable lands. Results of this study will provide a starting point to the development of an helpful decision maker tool, in order to select the most appropriate mitigation measures at local level.

Results of improvements at data availability at National level, would allow the results of this work to be extended to the entire Italian agricultural landscape.

We examined Chongqing municipality in China as a typical case to quantify its land use change associated with urbanization. Data on land use change were collected from Annual Reports of Chongqing Land Use Change Situation issued by the Chongqing Land and Resource & Housing Management Bureau. The results of this study revealed the relationships between river water quality and land use changes associated with urbanization. It is concluded that there is an urgent need to conduct comprehensive land use planning for protecting water quality in this rapidly developing megacity. This conclusion probably also applies to many other cities that are in a similar situation.

We present options for risk mitigation measures, which consider Swiss agricultural schemes and land-use. The planned procedure involves four main steps:

- Improvements of data availability at National level, would allow the results of this work to be extended to the entire Italian agricultural landscape.
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With GIS methods both datasets are spatially related. Although the dataset stem from different data models a good degree of consistency is achieved as both datasets are.

Information on the surface water network stem from the German authoritative topographic database ATKIS. For the.

In the web application the user can select a certain field on a detailed map, choose a crop to be treated and upload the pesticide applications to be tested. The field selection.

For the assessment a zone of 10m directly adjacent to the top of the water body embankment is analyzed in 1m steps.

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...to assess the aquatic and terrestrial risk of pesticides on field level...

...the use of pesticides in agriculture causes environmental risks that must be carefully managed. One overall goal of the current German National Action Plan (NAP) on the...
Explanatory power of peak and time-weighted averaged concentration for effects of pesticides as observed in semi-field experiments

M.L. Zattara, J.K. Deneve, P.A. Vant, D.J. Dröge

Wagenings University and Alterra Research Centre, Wageningen, Netherlands

Laboratory and semi-field experiments usually do not provide in the effects of time-variable exposure to aquatic organisms. This issue was emphasized in a recent EU ELINK workshop on linking exposure and effects to the aquatic risk assessment procedures for pesticides, and resulted in recommendations for addressing time-variable exposure, and when to use peak or time-weighted averaged (TWA) concentration. The aim of present study was to compare the effects as observed in cosm experiments with peak concentration of the exposure profile as well as the TWA21d concentration using different sensitivity endpoints. For this purpose, a literature review was performed of empirical PERF database which has been built by performing a review of freshwater model-ecosystem studies evaluating the effects of pesticides. Peak exposures of single and multiple application of pesticides were derived from the publications and their corresponding TWA21d concentrations were calculated. In order to allow comparison of studies with different insecticides, we expressed the exposure concentration as toxic units (TU). TU were calculated by dividing the concentrations evaluated in the cosm study by the Hazard Concentration 50% (HC50). Different grouped endpoints were selected from each microcosm and mesocosm study and responses were assigned to effect class. In case of peak exposure to the organophosphate insecticide chlorpyrifos, clear effects (Class 3) on "Microcrustaceans" are regularly reported at exposure concentrations below the range of 0.111 Peak-TU, slight effects (Class 2) are observed. However, if the concentration is reduced to 0.051 Peak-TU, these effects are no longer observed. The data presented for microcrustaceans and chlorpyrifos show a clearer dose-response relationship in case of TWA exposures, when compared to peak exposure. For this comparison, the effects of pesticides will be also be presented for other insecticides and they will also be aggregated for mode of actions and chemical groups.

Behavioural analysis on Daphnia magna exposed to different pollutants: possible contribution for water quality assessment

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EDF R&D-Laboratoire National d’Hydraulique et Environnement, Chatou, France

Behavioural endpoints are presented as function of concentration and time for each substance in order to understand changes in behaviour induced by toxicant and to predict the time of effect onset. Results are discussed with regards to possible utilisations and limitations of behavioural endpoints for early warning system in water quality control.

Assessment of tissue-specific accumulation and effects of cadmium in a marine fish fed contaminated commercially produced diet


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The aim of the study is (i) to assess how behavioural responses can be used as ecotoxicological endpoints and (ii) to compare the sensitivity of standard versus behavioural endpoints towards different endpoints from observation of daphnia behaviour, how to quantify the severity of these endpoints and finally how to use these endpoints in the context of environmental risk assessment. The aim of the study is (i) to assess how behavioural responses can be used as ecotoxicological endpoints and (ii) to compare the sensitivity of standard versus behavioural endpoints towards different endpoints from observation of daphnia behaviour, how to quantify the severity of these endpoints and finally how to use these endpoints in the context of environmental risk assessment. The aim of the study is (i) to assess how behavioural responses can be used as ecotoxicological endpoints and (ii) to compare the sensitivity of standard versus behavioural endpoints towards different endpoints from observation of daphnia behaviour, how to quantify the severity of these endpoints and finally how to use these endpoints in the context of environmental risk assessment.

Linking Selenium exposure to physiological, biochemical and morphological responses in three species of salmonids - use of weighted logistic regressions to compare sensitivity of endpoints

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Coal mining, uranium mining and irrigation of selenium (Se)-rich soils contributes to loading of Se into surface waters. Although Se is an essential element, it becomes toxic at concentrations slightly above those required for homeostasis. Fish are susceptible to Se toxicity and species-specific sensitivity to Se has been documented in adult fish and also for larvae, embryos and larvae. We have gathered physiochemical, biochemical and morphological data from several species of salmonids fishes (rainbow trout, brook trout, cutthroat trout) exposed to selenium either in mine pit lakes located on reclaimed coal mining sites, streams draining these areas, or in fish exposed to Se through diet or water in the laboratory. Specific sets of data, including plasma hormone concentrations, liver lipid peroxidation, liver GSH content, liver glycogen reserves, and condition in adult fish, were used for the study of endpoints (several number of malformations (craniofacial, spinal, edema and fin fold), weight and swim up success in fish larvae, are regressed against tissue Se using weighted logistic regression to identify response thresholds. Response thresholds are then compared to determine the most sensitive endpoint, for use in risk assessment and diagnosis of Se-caused adverse effects. (Funded by MITHE-SN, Alberta Conservation Association, NSERC).
a wide range of soil properties (pH, organic matter content, texture and cEC). In addition, toxicity of Pb was compared in 3 soils under 3 conditions: i) freshly spiked with PbCl$_2$, ii) freshly spiked, leached and pH corrected, and iii) freshly spiked and aged for 5 years. Variation in soil properties significantly explained variation in Pb toxicity for some endpoints (e.g., nitrification), but for other endpoints (microbial respiration, Folsomia candida reproduction) Pb toxicity was not affected by soil properties. Leaching and complexation increased Pb toxicity significantly, whereas aging increased higher toxicity in freshly spiked soils compared to soils equilibrated under field conditions. The data were used for the derivation of a refined correction factor for leaching and aging effects. This paper describes the implementation of the new data and bioavailability models in the terrestrial effects assessment and derivation of a PNEC$_{Pb}$ for Pb.

WE 129
Field validation of cell viability as a biomarker of metal exposure in soil invertebrates
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Invertebrates such as arthropods and mollusks are often used in toxicity assessments of metal polluted environments, as they are known to bioaccumulate metals. It is also known to exhibit various subcellular metal toxic effects as a result of bioaccumulation, for example reduced cell membrane integrity. In a previous study we investigated the potential use of the trypan blue exclusion assay to demonstrate effects of metals on hemocyte viability of woodlice. The aim of the current study is to test the field application of this technique. Millipedes (Ommatoiulus moreletii) and snails (Helix aspersa) were collected from a metal smelter, as well as from an unpolulated botanical garden, and were kept in the laboratory. The trypan blue exclusion assay was performed on these animals. The animals were then digested with nitric acid and metal concentrations determined with an ICP-AES. Results generally showed higher metal concentrations in animals from the metal smelter, with concomitant reduced cell viability. This suggests that cell viability, using the trypan blue exclusion assay, may possibly be successfully used within a suite of biomarkers, as a general indication of stress.

WE 130
Linking subcellular metal partitioning to the particulate and dissolved exposure pathways and chronic effect of copper in two deposit feeder organisms
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In toxicity assessment, increasing importance is being placed on determining tissue metal-effect relationships that result from environmental exposure. Toxic effects are often detected post-hoc, and cannot be linked to specific subcellular, or even cellular, events that may influence toxic potential. In this study we focused on two deposit feeder organisms, and examined how metal accumulation in tissues may be linked to subcellular partitioning of metals. We used a range of techniques to measure tissue partitioning and accumulation, and the subcellular metal exposure may be used to predict toxic effects at the tissue level, and ultimately determine the subcellular metal exposure that may result in toxic effects at the whole animal level. The results highlighted: i) strong correlation between total copper bioaccumulation and both POC-normalization copper concentration of the <63 μm sediment fraction and copper concentration in overlying water for both organisms (M. plumulosa r$_{0.07}$=0.87, p<0.001; r$_{0.07}$=0.84, p<0.001; T. deltoidalis r$_{0.07}$=0.93, p<0.001; r$_{0.07}$=0.96, p<0.001); ii) significant correlation between the metabolically available fraction of copper and the particulate copper concentration in sediment for both organisms (M. plumulosa r$_{0.07}$=0.57, p<0.001; T. deltoidalis r$_{0.07}$=0.67, p<0.001); the correlation was quite weaker for M. plumulosa between the metabolically available fraction and copper concentration in the overlying water (r=0.44, p=0.05, df=9) but stronger for T. deltoidalis (r=0.92, p<0.001); iii) strong linear relationship between the compartment of copper biologically detoxified within T. deltoidalis and both the % POC/silt normalized copper concentration and copper concentration in water phase (r=0.83, p<0.001; r=0.83, p=0.01) between subcellular effect and metabolically available fraction for M. plumulosa. Copper associated with the particulate phase has been demonstrated to be the major exposure route and potential cause of the toxicity for M. plumulosa. However, in T. deltoidalis, particulate and dissolved exposure routes seem to play both an important role in copper uptake. These results provide further insight into the interaction between tissue residues and chronic effects.

WE 131
The impact of contamination by phospophygpton on the structural and functional diversity of the soil microbial community in a deposition area (Sfax, Tunisia)
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The phosphate industry is an important economic activity in Tunisia. Nevertheless it originates several tons of phosphogypsum (PG) (5 tons of PG per 1 ton of phosphoric acid). The objective of this study was to investigate the impact of the production of phosphorus fertilizers by a wet-acid method. Usually PG is dumped in stockpiles, exposed to weathering conditions and contributing for the spread of contaminated dusts, causing serious environmental and health problems. In this work we studied the impact of contamination near the PG pile area (Sfax, Tunisia) on the structural and functional diversity of the soil microbial community. With this purpose we choose five transects in the area, where four soil samples were collected five cm from a chemical fertilizer on one of these transects (Topsoil). A dated soil sample without fertilizer was collected as a reference from another transect (Topsoil). The samples were then digested with nitric acid and metal concentrations determined with an ICP-AES. Results generally showed higher metal concentrations in animals from the metal smelter, with concomitant reduced cell viability. This suggests that cell viability, using the trypan blue exclusion assay, may possibly be successfully used within a suite of biomarkers, as a general indication of stress.

WE 132
Exposure of honey bees and other pollinating species to pesticides
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Plant pests and diseases (PPDs) (also called pests and diseases) are part of modern crop management practices. Prior to the placement of PPP on the market and their use an evaluation of the risks posed to the environment is mandatory worldwide requiring an assessment of the impact of these products on the agricultural environment, and among others on arthropod and pollinating species. The exposure pathway of honey bees can occur both the hazard (toxicity) of the compound and also the potential exposure to the organism is then considered. The risk assessment usually follows a tiered approach whereby products of low toxicity and low risk are rapidly excluded; whereas products with a potential to harm honey bees are progressed to higher and more realistic tiers of evaluation. Consequently, it is usually not necessary to generate extensive and elaborate measures of toxicity and exposure in tier 1 assessments. Many PPP are typically applied either by spray to the above ground part of plants or to soil either directly (by spray or granules) or into the soil as treated seed. A third method of plant injection is important for the protection of trees. The method and timing application will affect if bees are exposure and if so by what routes. The physiochemical properties can also impact exposure which may impact the distribution of the substance within plant and also the exposure site. In this paper, different exposure scenarios are developed with respect to method and timing of application also in relation to the properties of the substance. Various scenarios are described where bees and other pollinators can be exposed along with an indication of possible residues levels present in matrices of relevance to bees. Explanations of the exposure scenarios due to individual applications and soilseed treatment uses are given and way in which be can be used and link to risk assessment presented. By establishing clear guidance for exposure of pollinators to PPP a more informed risk assessment process can be developed and utilized.

WE 133
Environmental assessment of a low molecular weight Phthalate Ester - DEP (Diethyl Phthalate)
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The phosphate industry is an important economic activity in Tunisia. Nevertheless it originates several tons of phosphogypsum (PG) (5 tons of PG per 1 ton of phosphoric acid) which is a waste-by product of the production of phosphorus fertilizers by a wet-acid method. Usually PG is dumped in stockpiles, exposed to weathering conditions and contributing for the spread of contaminated dusts, causing serious environmental and health problems. In this work we studied the impact of contamination near the PG pile area (Sfax, Tunisia) on the structural and functional diversity of the soil microbial community. With this purpose we choose five transects in the area, where four soil samples were collected five cm from a chemical fertilizer on one of these transects (Topsoil). A dated soil sample without fertilizer was collected as a reference from another transect (Topsoil). The samples were then digested with nitric acid and metal concentrations determined with an ICP-AES. Results generally showed higher metal concentrations in animals from the metal smelter, with concomitant reduced cell viability. This suggests that cell viability, using the trypan blue exclusion assay, may possibly be successfully used within a suite of biomarkers, as a general indication of stress.
these organisms. In summary, DFP does not pose a significant risk to the aquatic or terrestrial environment in terms of PBT or ED.

WE 134
Toxicokinetics model for an insecticide in rats: implications for higher-tier risk assessment
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The current risk assessment for mammals is based on oral exposure measurements. Exposure to a chemical does not mean, however, that all of the dose will be bioavailable. Bioavailability, i.e., fraction of dose that reaches the systemic circulation or is made available at the site of physiological activity, as well as toxicokinetics (e.g., absorption, distribution, metabolism, and excretion), plays a crucial role in this internal concentration which makes an effect. Internal concentration of the net result of absorption, distribution, metabolism and excretion, and the toxicokinetic (TK) model is a mathematical description of these processes. We used data for an insecticide. The toxicokinetic model was parameterised using absorption, tissue distribution and excretion from a rat study with 14C-labelled material. The number of compartments in the model and the complexity of the processes of absorption and elimination were also investigated using these data. Feeding rate scenarios and avoidance of contaminated food were taken into account in the model because they may influence the concentration of chemical in the body and the risk for animals living in natural environments.

High variability in kinetic parameters between individuals meant it was necessary to test the effects of different combinations of maximum and minimum values for absorption ka and elimination ka rate constants on the change of the concentration in the bloodstream as a function of time for each exposure scenario (i.e., LD50 eaten as a bolus dose or according to three different feeding patterns). Maximum internal concentrations (CMaxBody) for all studied scenarios were compared. Results showed that, irrespective of the scenarios, higher variability in kinetic parameters, higher probability for the error in the estimated rate constants on the change of the concentration in the bloodstream resulted in more variability in kinetic parameters and in the error in the estimated

WE 135
Relevance of drinking water as a source of human exposure to bisphenol A
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The fraction of drinking water measurements were conducted to characterize the occurrence and concentrations of BPA. BPA has been detected in a small fraction of drinking water and source water samples. The fraction of drinking water measurements was less than the detection limit is 95%, 48%, and 41%, for North America, Europe, and Asia, respectively. The highest mean measured drinking water concentration in Europe, Asia, and North America were 0.009, 0.014, and 0.317 microg/L, respectively. The median and 95th percentile concentrations of BPA in Asian drinking water were 0.026 and 0.19 microg/L, while the median and 95th percentile concentrations in North America and Europe are limited by detection limits. In surface water/surface water, the median and 95th percentile detection limits are: <1 and 1 microg/L for North America, <0.006 and <5.1 microg/L for Europe, and the median and 95th percentile detection limits are: <0.015 microg/L for Asia. If the median and 95th percentile detection limits are not available for all samples, the oral reference doses (RIDs) for human exposure to BPA have been derived by several agencies, including the US National Toxicology Program, the US Food and Drug Administration (FDA), and the European Food Safety Authority. In 2009, the 90th percentile concentration for drinking water is 0.026 and 0.099 microg/L, respectively, median and 95th percentile intakes of BPA due to ingestion of drinking water is 0.011 and 0.040 microg/L for Europe and Asia, respectively. In summary, DEP does not pose a significant risk to the aquatic or terrestrial environment in terms of PBT or ED.

WE 136
Urinary bromphenols phase II conjugates: a novel human exposure biomarker for PBDEs
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We synthesized a number of glucuronide and sulfate conjugates of BDE congeners in human blood plasma. We hypothesized that these Phase II PBDE metabolites of BDE congeners are also present in human urine and their levels can be used to correlate the extent of exposure to PBDEs. If this hypothesis is validated, a more reliable population exposure assessment for PBDEs can be developed through the measurement of the glucuronide and sulfate conjugates of bromophenols in human urine. To examine this hypothesis, we synthesized dibromophenol glucuronide and sulfate conjugates of BPs and studied the correlation between their levels in human urine and those of their apparent parent BDE congeners in human blood plasma.

WE 137
Improvement of the human respiratory risk assessment by including alkylated PAHs into the analyses
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Polycyclic aromatic hydrocarbons are very important environment pollutants. They are generated during various combustion processes and can be found at high levels at all environmental compartments. Many of them may pose severe health risk. They exhibit induction of aryl hydrocarbon receptor (dioxin-like toxicity) and either estrogenic or antiestrogenic effects on the body. For the evaluation of PAHs are often monitored in all environments. Their alkyl derivatives are often produced together with the parent compounds. Even though their environmental levels are often comparable to those of PAHs and they can pose similar health risks, levels of alkylated PAHs are rarely monitored. This paper is focused on the assessment the dioxin-like activity and estrogenicity of alkylated PAH using cell lines. Drosophila melanogaster (melanogaster) cells (H41B-luc) after treatment with different PAHs. The dioxin-like activity of alkylated PAHs was assessed in these cells. To evaluate the estrogenicity of alkylated PAHs, the affinities to the human estrogen receptor alpha (ERα) were determined. These PAHs were able to activate the estrogen receptor in the human cell line (MCF-7). In conclusion, alkylated PAHs can be considered as a new group of estrogens. These results may be important for the risk assessment of PAHs in the environment.

WE 138
Chemical synthesis, characterization and analytical development of bromophenols phase II conjugates as human exposure biomarker for PBDEs
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The occurrence of Phase II metabolites of selected BDE congeners in urine of mammalian animal models has already been well-established by numerous pharmacokinetic and toxicokinetic studies. These metabolites are mainly glucuronide and sulfate conjugates of dibromophenols (DBPs) and tribromophenols (TPBs). To the best of our knowledge, there is no literature report on the occurrence of these Phase II PBDE metabolites in human urine. It would be ideal if these glucuronide and sulfate conjugates of bromophenols (BPs) in human urine can be used as exposure markers for PBDEs. The aims of this study is to synthesize, characterize and purify selected dibromphenyl- and tribromphenyl glucuronide and sulfate conjugates as authentic and calibrated standards for the development of appropriate LC-MS/MS analytical method to quantify these dibromphenols and tribromophenols in human urine samples. The structures of target glucuronide and sulfate conjugates are shown in the Figure 1. Authentic standards of the target glucuronide and sulfate conjugates of 2,4-DBP and
2,4,6-TBP were synthesized and purified by in-house procedures. These four BP conjugates were fully characterized by high-resolution mass spectrometry, elemental analysis and 1H NMR spectroscopy. Their purity was validated by LC-MS with triple quadrupole tandem mass spectrometer. Also, the stability of these conjugates in human urine under various preservation conditions were investigated.

WE 139
A survey of the traditional food diet that may contribute to enhanced soil ingestion in a Canadian first nation community
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An ethno-cultural survey was conducted of the Xeni Gwet’in First Nations community living in the Nemiah Valley, approximately 230 km west of Williams Lake, British Columbia. The purpose of this study was to assess and document the traditional food consumption and activities of the community that may lead to enhanced exposure to soil. The potential for ingesting soil from consuming traditionally-prepared food was also assessed through the analysis of traditional food items for tracer elements commonly found in soils. The community was observed to consist mainly of fish and big game, and was supplemented by berries and roots. Outdoor cultural gatherings, hunting and food gathering trips and sporting events, with their attendant potential for enhanced soil exposure, was observed to be an important facet of community life. The survey concluded that a significant portion of the Xeni Gwet’in practice a lifestyle similar to the subsistence lifestyle of other indigenous communities, where soil exposure scenarios in the traditional diet have been documented. Soil exposure potential of food items consumed at such food, specifically roots, may have substantial amounts of soil adhering to them that would result in soil ingestion in the order of approximately 30 g of soil per 100 g serving.

WE 140
Ecotoxicity, cytotoxicity and genotoxicity of PM10 air pollution. Correlation with chemical pollutant levels
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Air is composed by multiple environmental chemicals pollutant mixtures that may pose important adverse health effects, not only because of the effects of individual substances, but also as a result of the potential synergies/antagonisms among them. Despite that, and because of the difficulties to assess the hazard potential of these mixtures, regulation measures to control the levels of pollutants are traditionally focused on determining the levels of contaminants individually. Air inhalation is known to be an important exposure pathway to some chemicals, such as heavy metals, organic pollutants and particulate matter. Correlation between high air pollution and adverse effects on human populations has been largely recognized, but the toxicology of these adverse effects has not been clarified. In recent years, some surveillance programs have been performed in order to monitor the air quality in industrial (close to incineration and cement plants) and urban areas in Catalonia (Spain). Data on the concentrations of metals (As, Cd, Cr, Hg, Mn, Ni, Pb, Ti, U, and V), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) as well as particulate matter (PM10), obtained by means of hourly sampling devices, across sampling devices, were measured. The objectives of the present study were: i) to develop and validate a good methodology to obtain these results, ii) to study the metal levels in PM10 samples in order to assess toxicity tests, and ii) to evaluate the most appropriate toxicity tests to apply in this specific samples. Aqueous and organic extractions of quartz fiber filters, where PM10 had been retained, were performed in order to evaluate the toxicity of polar and non polar fractions, respectively. Ecotoxicity of PM10 was performed by Microtox® bioassay utilizing Vibrio Fischeri. Cytotoxicity and genotoxicity were determined by MTT Assay and Comet Assay, respectively, using human lung epithelial cells (A549) as target cells. Preliminary results showed that QFF extraction with a mild acid is a good methodology to obtain significant toxicity results. These air samples presented effect at cellular level but they did not present effect at genic level. Both Microtox test and MTT assay were appropriate in order to assess the ecotoxicity and cytotoxicity of air samples.

WE 141
Consumers exposure to particle-bound PAHs at night markets in Taiwan
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This study conducts the health risk assessment for consumers exposure to carcinogenic PAHs at night markets in Taiwan. In Taiwan, there are more than 300 night markets that are popular and famous for offering a wide range of local delicacies and traditional snacks. Night market is a very dense outdoor cooking environment. When the concentrations of cooking hoods are high, the cooking fumes can be harmful to both the cooks and the consumers present in the night markets. Real-time PAHs concentration detector (Grimm 1000) was used to collect particle-bound PAHs with 16 different PAHs from different channels. 13 samplings were conducted during 27 October, 2011 to 22 November, 2011. It includes 5 weekday night, 5 weekend night, and 3 background samplings. Exposure assessment were assumed that the consumers will spend 40 minutes eating dinner and 20 minutes shopping. Two different exposure concentration were estimated too. This study also used toxic equivalent factors of U.S. EPA to estimate the potential carcinogenic (Bapq) for consumers exposure. The most dominate PAHs concentration were in 2.0-3.0 μm with 14.56% for total PAHs concentration. In weekday, the average PAHs concentration were 280.14 ng m^-3 and 178.73 ng m^-3 for eating period and shopping period, respectively. In weekend, the average PAHs concentration were 426.68 ng m^-3 and 298.78 ng m^-3, respectively. Results indicated that more high concentration than shopping area, it may caused by cooking fumes emitted to neighboring area. Results show that the total average Bapq were 83.32 ng m^-3 and 129.90 ng m^-3 for weekdays and weekend, respectively. Respiration and diet exposure to cooking emissions in Consumer at night markets is of health concern.

WE 142
Metal exposure exacerbates influenza infection-related lung function decrement risk for smokers
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Malnutrition is a main health issue associated with overweight, and obese groups which are concerned with the increases in the risks of morbidity and mortality worldwide. Metal particle exposure and influenza infection can both lead to lung function deterioration. The objectives of this study were three folds: (i) to construct the dose-response relationship between metal particle exposure and lung function deterioration for Fe(II)-metal and SmFe(III)-metal with various body mass index (BMI) groups, (ii) to build up the influenza infection-related dose-response profile of forced expiratory volume in 1 second (FEV1) and respiratory symptoms scores (RSS), and (iii) to assess the potential risks posed by smelting metal dust exposure and influenza infection-related RSS exacerbations among different BMI groups. The results showed that the highest metal dust concentrations for Fe(II)-metal and SmFe(III)-metal exposure induced FEV1 reducing rate of 0.59 and 1.11 mg 1-1, respectively. The estimated BMI-specific FEV1 were 3.41-3.56, 3.56-3.60, 3.44-3.64, and 2.70-3.44 for underweight (BMI<18.5), normal (18.5≤BMI≤24), overweight (24≤BMI≤27), and obese (BMI≥27) groups, respectively. For SmFe(III)-metal smokers, the metal exposure and influenza infection exacerbated RSS with a decrease of risk at 0.5 (ER0.5) were estimated to be 0.25-0.35, 0.21-0.29, 0.26-0.35, and 0.33-0.39, for underweight, normal, overweight, and obese groups, respectively. For SmFe(II)-metal smokers, the estimated exacerbation RSS at ER0.5 were 0.24-0.36, 0.21-0.24, 0.24-0.34, and 0.39-0.43, respectively, for underweight, normal, overweight, and obese group. This study provides a novel probabilistic risk assessment framework to better assess occupational health risk given metal dust exposure and influenza infection.

WE 143
Novel approaches for static mesocosm studies with herbicides: linking modelled exposures with the reality of flowing water and other challenges
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A great deal of advance thought and planning is required for many substances before initiating a mesocosm study. Otherwise there is a risk of conducting a study that is of limited regulatory use. Here we will give advice and considerations for mesocosm studies with herbicides with long aquatic half-lives. This will be illustrated using example state-of-the-art mesocosm studies. It is hoped that this paper will advance mesocosm study design and offer advice to those considering a mesocosm study. Each study required special consideration of the exposure profile in the static mesocosm system versus the reality of flowing water, and a novel design following the principles of E-Link was used.

Along with providing conservative exposure estimates, a number of other technical challenges were faced regarding the experimental design and practical conduct of the studies. The key issues faced will be discussed to drive discussion on alternative approaches for mesocosm testing.

WE 144
Time-dependent toxicity of pesticides and other toxicants: implications for a new approach to risk assessment
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A fundamental goal of toxicology is to determine safe levels of exposure to potentially poisonous substances for humans and the environment. Traditionally, safe levels of a chemical have been derived from the non-observable effect level (NOEL) estimated in laboratory toxicity bioassays with organisms which are representative of certain taxa. This approach is inconsistent with the view that health results from a host of factors - both toxic and non-toxic - that interact in a non-linear manner. It also assumes that toxic chemicals do not have any effect on a population of organisms at very low concentrations. Recent developments in ecotoxicology, however, suggest that some toxicants can produce effects at any concentration level provided their exposure time is sufficiently long. Consequently, risk assessment of these chemicals, which includes neonicotined insectsicides, some carcinogenic substances and certain metallic compounds, may require entirely new approaches. Because the relationship to toxicity is a function of time, these relationships at arbitrarily fixed exposure durations which are supposed to reflect 'acute' or 'chronic' time scales. This approach measures the proportion of all exposed individuals responding by the end of those fixed exposure times. However, the endpoint values obtained this way cannot be linked to make predictions for the wide range of exposures encountered by humans or in the environment, thus leading to serious underestimates of actual risk. In order to overcome this handicap, an increasing number of researchers are using a variant of the traditional toxicity testing protocol which includes time to event (TTE) methods. This TTE approach measures the times to respond for all individuals, and provides information on the acquired doses as well...
WE 145  Linking fluctuating exposure concentrations to effects: a retrospective and a prospective case study  R. A. L. W.  EAWAG, Dubendorf, Switzerland

Two case studies, a retrospective and a prospective case study, are presented in which toxicokinetic-toxicodynamic (TKTD) modelling was applied to predict effects of fluctuating concentration on aquatic organisms.

In the retrospective case study the prediction of toxicity over time was separated from the extrapolation from one to many species and from acute to sublethal effects. A toxicokinetic-toxicodynamic (TKTD) model predicted toxicity caused by fluctuating concentrations of diazinon, measured by time-resolved sampling over 108 days from three French golf courses. Temporal fluctuation factors were calculated to quantify variable toxicity among species and effect types based on available toxicity data, while correcting for different test durations with the TKTD model. Sampling from the distribution of extrapolation factors and prediction of time-resolution toxicity with the TKTD model facilitated subsequent calculation of the risk of undesired toxic events. Approximately one-fifth of aquatic organisms were at risk and fluctuating concentrations were more than their averages. Contribution of urban and agricultural sources of diazinon to the overall risk varied.

The second case study was part of the regulatory risk assessment of the product. The objective was the prediction of mortality towards fish under fluctuating and pulsed exposure. TKTD modeling was applied to predict survival of five fish species under fluctuating exposures to the pesticide. The General Unified threshold model for Survival (GUTS) was used for TKTD modelling after calibration on standard acute toxicity data (96h-LC50 tests). TKTD model parameters were calculated with the TKTD model to be used with the TKTD model to facilitate subsequent calculation of the risk of undesired toxic events. Approximately one-fifth of aquatic organisms were at risk and fluctuating concentrations were more than their averages. Contribution of urban and agricultural sources of diazinon to the overall risk varied.

WE 146  Ecological impacts of time-variable exposure regimes on pesticide residues on freshwater communities  M. Zafar1, R. P. van Wijngaarden2, J. D. Belgers3, J. D. J. B. Hoeksema4, R. P. A. van Wijngaarden1 1Wageningen University and Alterra Research Centre, Wageningen, The Netherlands 2WUR, Alterra, Wageningen, the Netherlands 3Akvaplan-niva, Tromsø, Norway 4INRA, Rennes, France

We provide evidence linking emerging environmental contaminants with health disruption of fish, based on ecological studies, chemical analysis, controlled laboratory experiments, and physiological/biochemical parameters. Emerging contaminants detected in the Southem Alberta rivers include pharmaceuticals, plasticizers, and other organic compounds. To investigate a link between exposures to environmental contaminants and adverse health effect in fish, we performed field studies and collected long-term data as a suitable sentinel species abundant in most locations. The Oldman River runs through areas with significant agricultural and feedlot activities as well as a number of key fish species, including northeast arctic cod and haddock. The Oldman River runs through areas with significant agricultural and feedlot activities as well as a number of key fish species, including northeast arctic cod and haddock. The Oldman River runs through areas with significant agricultural and feedlot activities as well as a number of key fish species, including northeast arctic cod and haddock. The Oldman River runs through areas with significant agricultural and feedlot activities as well as a number of key fish species, including northeast arctic cod and haddock.

WE 147  Structural and functional effects of conventional and low pesticide input and oilseed rape crop protection programmes in outdoor aquatic mesocosms  A. Aubert1, M. Roucaute1, T. H. Caquet2, B. Réal1, N. Surylèc3, I. Dibus4, A. Togola5, D. Azam5, L. M. Ladagic2

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This study was designed to evaluate the benefit to aquatic ecosystems of reducing pesticide inputs in crop protection programmes. Models of pesticide transfer (Ganzlemeier curves for drift; MACRO for drainag; PRZM for runoff) were used to estimate fluxes and dates for transfer for each substance under conditions of hydromorphic soils of Northern France. Two contrasting pedoclimatic conditions were considered, which correspond to drained soils of north-western France (Jaillière) and of the northeast of the Parisian Basin (Brie). Each condition was associated to two wheat and oilseed rape crop protection programmes (hereafter named ‘Conventional’ and ‘Low pesticide inputs’ which include product substitution and application rate reduction) representative of actual agricultural practices. Model estimates of the concentrations and dates at which pesticide mixtures enter water bodies were used to contain outdoor pond mesocosms (9 m3). Three mesocosms were used for each treatment and four ponds were kept for the Conventional programme and two for the Low pesticide input programme. The treatment levels of single and four applications were fixed from the combination of two pesticide inputs from the Conventional programme, which are placed in between the chronic treatment regimes, indicating that for long-term effects of chlorpyrifos the TWA is more important than the peak concentration. The predicted toxicity with the TKTD model predicted toxicity caused by fluctuating concentrations of diazinon, measured by time-resolved sampling over 108 days from three French golf courses. Temporal fluctuation factors were calculated to quantify variable toxicity among species and effect types based on available toxicity data, while correcting for different test durations with the TKTD model. Sampling from the distribution of extrapolation factors and prediction of time-resolution toxicity with the TKTD model facilitated subsequent calculation of the risk of undesired toxic events. Approximately one-fifth of aquatic organisms were at risk and fluctuating concentrations were more than their averages. Contribution of urban and agricultural sources of diazinon to the overall risk varied.

WE 148  Emerging contaminants cause adverse health effects in native species of fish in Southern Alberta Rivers  H. A. Henderson1, C. Maier2, A. Zatke 3, J. Jordan 3, A. Weljie4, L. J. Jackson

1University of Calgary, Calgary, Alberta, Canada 2University of Calgary, Calgary, Alberta, Canada

The present study was carried out to investigate the species and adverse effects of emerging contaminants in the Bow and Oldman Rivers in Southern Alberta, Canada. We provide evidence linking emerging environmental contaminants with health disruption of fish, based on ecological studies, chemical analysis, controlled laboratory experiments, and physiological/biochemical parameters. Emerging contaminants detected in the Southem Alberta rivers include pharmaceuticals, plasticizers, and other organic compounds. To investigate a link between exposures to environmental contaminants and adverse health effect in fish, we performed field studies and collected long-term data as a suitable sentinel species abundant in most locations. The Oldman River runs through areas with significant agricultural and feedlot activities as well as a number of key fish species, including northeast arctic cod and haddock. The Oldman River runs through areas with significant agricultural and feedlot activities as well as a number of key fish species, including northeast arctic cod and haddock. The Oldman River runs through areas with significant agricultural and feedlot activities as well as a number of key fish species, including northeast arctic cod and haddock. The Oldman River runs through areas with significant agricultural and feedlot activities as well as a number of key fish species, including northeast arctic cod and haddock.

WE 150  Symposium - a risk assessment tool for the oil industry in the Lofoten/Barents Sea  S. J. Moe1, D. Howell2, J. Jolyon3

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There is increasing interest in oil exploration in the Norwegian arctic, in particular around the Lofoten islands off northern Norway. This area is also the spawning ground for a number of key fish species, including northeast arctic cod and haddock, Norwegian spring spawning herring. The narrow coastal shelf contains the fish larvae into a small area during the early stage of their life cycle. It is important to utilize the best available knowledge about the ecosystem in order to assess the risks of oil spill effects on the environment. We provide evidence linking emerging environmental contaminants with health disruption of fish, based on ecological studies, chemical analysis, controlled laboratory experiments, and physiological/biochemical parameters. Emerging contaminants detected in the Southem Alberta rivers include pharmaceuticals, plasticizers, and other organic compounds. To investigate a link between exposures to environmental contaminants and adverse health effect in fish, we performed field studies and collected long-term data as a suitable sentinel species abundant in most locations. The Oldman River runs through areas with significant agricultural and feedlot activities as well as a number of key fish species, including northeast arctic cod and haddock. The Oldman River runs through areas with significant agricultural and feedlot activities as well as a number of key fish species, including northeast arctic cod and haddock. The Oldman River runs through areas with significant agricultural and feedlot activities as well as a number of key fish species, including northeast arctic cod and haddock. The Oldman River runs through areas with significant agricultural and feedlot activities as well as a number of key fish species, including northeast arctic cod and haddock.
potential development in the area. A range of separate models exist for the distribution of petroleum compounds, ecotoxicology, plankton, larvae, and fish in the region. The SYMBIOSES project aims to integrate these into a single tool, which will examine the effects on the major fish stocks of possible oil-spill impacts on larval survivorship, and thus evaluate the combined effects of fisheries and petroleum-related activities in the area. Quantitative measures of uncertainty in the ecosystem and ecotoxicology simulation results will be explored using Bayesian probability networks. The model can then be used alongside analyses of other risks (sublethal effects, local ecosystem, seabirds, etc.) to give improved risk assessments for oil developments.

WE 151

Applying dynamic energy budget theory to estimate the impact of oil components in an Arctic food chain


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The Lofoten and Vesteraalen region in Northern Norway is a biologically rich area with one of the most important breeding grounds for North East Arctic cod. After oil and gas reserves were discovered a debate has been ongoing in Norway whether the risks of oil and gas exploration on this biologically rich area are acceptable. A decision on exploration or not is postponed oil 2013 and in the meantime efforts are made to quantify these risks.

A 3-year project called SYMBIOSES has been initiated to develop a holistic, integrated modeling framework for ecosystem-based risk and impact assessments of oil components, with a special focus on the effects on fish stocks. In this framework, existing models describing oceanography, oil/chemical fate, the dispersal and growth of zooplankton (the main food of fish/larvae) and fish/larvae dynamics are coupled and extended with ecotoxicological algorithms that assess effect of oil on the key parameters of these models.

The Dynamic Energy Budget theory (DEB) will be applied to develop algorithms for the impact of oil components on the life-history processes growth, development and mortality of zooplankton. Experimental data on these life-history processes will be used to parameterize DEB. DEB is a mechanistic theory based on the principle that mass and energy are conservative quantities (one of the few hard laws available in biology), energy conversion process leads to dissipation, and mass and energy transport occurs across surfaces. DEB describes the energy pathways in individuals. The DEB model is developed as a nonspecies-specific metabolic model that allows differences between species to be reduced to differences in the set parameter values. The DEB model is tested for many species and deviations are rare. The DEB theory can thus be used to translate results of toxicity tests on reproduction, growth, development and mortality on the energy flow within the organism, and gives mechanistic insight in how a change in growth affects a change in reproduction and development, treating them as dependent. Furthermore, the mechanistic insight makes it possible to extrapolate results beyond the range of tested conditions, and include effects of multiple toxicants, and other factors such as temperature and food conditions.

This paper describes the proposed design plan for the ecotoxicology algorithms and presents preliminary results.

WE 152

Delayed effects of acute oil exposure on Calanus finmarchicus (Gunn) reproduction

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Delayed effects of acute oil exposure to dispersed oil on reproduction were assessed. Following a 96 hrs exposure to three concentrations of oil dispersions (0.25, 2.5 and 25 mg/L) generated from a North Sea crude oil, several reproductive endpoints (offspring production rates, egg hatching success, female reproduction participation, nauplii development) were studied in Calanus finmarchicus. A delay in the initiation of reproduction was observed for the group exposed to the highest dispersion concentration accompanied by a significant decrease in the number of females participating in reproduction was observed. However, a subsequent compensatory reproduction in the higher concentration group after a 10-day recovery period was observed. After 10 days post exposure no differences in egg production rates or hatching success were found between controls and oil-treated copepods. At the end of the experiment, the developmental stage distributions of offspring were comparable in both groups, suggesting reproduction rates higher than controls, after a 10-day recovery period was observed. 13 days post exposure no differences in egg production rates or hatching success were found between controls and oil-treated copepods. At the end of the experiment, the developmental stage distributions of offspring were comparable between all groups. The results indicate that, although a short-term exposure delays reproduction, copepods are capable of reproducing viable offspring after cessation of exposure to real life, such delays may have, however, ecological consequences due to relatively short periods of food accessibility (algal bloom) in cold waters. These aspects are important when assessing ecological consequences following an acute oil spill to Arctic and sub-Arctic marine environments.

WE 153

Rational application of chemicals in response to oil spills may reduce environmental damage

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Oil spills, for example those due to tanker collisions and groundings or platform accidents, can have huge adverse impacts on marine systems. The impact of an oil spill at sea depends on a number of factors, such as spill volume, type of oil spilled, environmental conditions, and proximity to environmentally, economically, or socially sensitive areas. Oil spilled at sea threatens marine organisms, whole ecosystems, and economic resources in the immediate vicinity, such as fisheries, aquaculture, recreation, and tourism.

Adequate response to any oil spill to minimize damage is therefore of great importance. The common response to an oil spill is to remove all visible oil from the water surface, either mechanically or by using chemicals to disperse the oil into the water column to biodegrade. This is not always the most suitable response to an oil spill, as the chemical application itself may also have adverse effects. In this article we discuss advantages and disadvantages of using chemical treatments to reduce the impact of an oil spill in relation to the conditions of the spill. The main characteristics of chemical treatment agents are discussed and presented within the context of a basic decision support scheme.

WE 154

Prediction and effects of oil dispersant application on marine shrimp in Tokyo Bay

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Heavy oil spill accidents that are happening in the world. Spilled oil attacked coastal area and adversely affected on aquatic organisms. During oil spill, dispersants have been applied to disperse spilled oil quickly. However, one cannot clarify whether the application of dispersant to spilled oil is an effective method or not in the environment.

In this study, the distribution of virtual spilled oil in Tokyo Bay, where many boats as well as tankers cruise, was examined by a model using a decision making process tool for oil pollution on GIS (DOG). The oil concentrations in the water and their risks on marine fish were predicted based on the water accommodated fraction (WAF) toxicity on organism and the concentration of dispersed oil by dispersants and lead to increase of toxicity on concentrations by disperse organisms. In addition, the risk of dispersed and oil spilled oil will be increased in the area of small exchange of sea water, while the risk will be decreased in the area of large exchange of sea water. Furthermore the strong wind is expected to disperse spilled oil greatly, thus the oil distribution can be small between application and no application of dispersant

WE 155

Pilot scale application for monitoring and optimization of petroleum hydrocarbon bioremediation

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Petroleum contamination of sandy soils is a severe environmental problem in Libya but relatively little work has been carried out to optimize how bioremediation of such poor soils can be carried out.

The purpose of this research is to determine the potential for bioremediation of hydrocarbon-contaminated soil obtained from oil refinery field, Libya. Hydrocarbon degrading microbes are present in the soil and that nutrient addition could increase hydrocarbon transformation. Therefore it was decided to attempt pilot-scale field studies using biodegradation techniques to treat contaminated oil. Plots containing 500 kg soil (triptichs) (contaminated soil diluted with clean soil 50% volume) were set up containing different nutrient levels Urea + NPK to obtain a C:N:P ratios 100:10:1, and monitored for 90 days. Hydrocarbon levels, microbial numbers and toxicity assessment (using luminescent microbial based tests) were determined throughout the experiment. Hydrocarbon levels, 731 - 22 345 mg/kg, and diluted soil 16 000 -17 000 ppm. Although all the land treatment units revealed a significant hydrocarbon reduction over time, the highest reduction on hydrocarbon obtained was 66 % on LTU1 ratio (100:10:1) and 63% on LTU5 diluted soil amended with Urea + NPK ratio (100:10:1) respectively, whereas the lowest reduction obtained 34% was on LTU4 control diluted soil. This suggests that the addition of nutrients increased the rate of hydrocarbon bioremediation.

The percentage of oil inhibition obtained by this assay was in good correlation with the reduction in total petroleum hydrocarbon determined, probably because both nutrient ratios used representing the right amendment needed.

WE 156

Influence of environmental parameters on oil spill effects using Taguchi experimental design methodology

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SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
of Ecotoxicology at the Regional University of Joinville. Even in small concentrations, 100% mortality of the organisms occurred after 2 hours of exposure. It was suggested that

Seven petroleum oils covering a representative range of commercially available products were tested. Dynamic tests at laboratory scale were performed to study 41 polycyclic aromatic hydrocarbons (PAHs) and derivates, among them 16 EPA priority pollutants. The coupling of the stir bar sorptive extraction (SBSE) and high performance gas chromatography mass spectrometry (HPGC-MS) was used. Limit of quantification were lower than 1 ng/L for each compounds. Experiments were performed according to Taguchi's design methodology. Four parameters were selected: temperature, suspended solid quantity, salinity, oil/water surface ratio. Each of these parameters was set at two levels in order to be represented by 8 different mixtures of oil and water were introduced into the oil/water interface. Water was allowed to disperse on the surface before starting a gentle magnetic stirring for 2h without vortex. The beaker was placed in a thermostatic chamber and darkness to avoid PAHs photodegradation. A large variation in composition of the water soluble fraction depending on oil type was highlighted. The chemical profile of the water soluble fraction was unlike that of the parent oil, but contained water-soluble compounds (naphthalene, anthracene) which constitute the major part of the water soluble fraction. For Heavy fuel and Jet, all parameters have been taken into account. For Bitumen, only suspended-soldid quantity can be neglected. For Domestic fuel, Gasoline, Diesel, salinity and suspended-solid quantity can be neglected. For all oils, the water soluble fraction was minimised with low temperature. For all oils except, Bitumen and Heavy fuel, the water soluble fraction was minimised with high surface ratio.

WE 157

Haptophytos and stenoses in recent sediments of the Continental Shelf of Yucatan, Mexico

E.C. Chan, V.M. Ceja, G.B. Gold

Center of Research and Advanced Studies of IPN, Merida, Mexico

Oil spills covering a representative range of commercially available products were tested. Dynamic tests at laboratory scale were performed to study 41 polycyclic aromatic hydrocarbons (PAHs) and derivates, among them 16 EPA priority pollutants. The coupling of the stir bar sorptive extraction (SBSE) and high performance gas chromatography mass spectrometry (HPGC-MS) was used. Limit of quantification were lower than 1 ng/L for each compounds. Experiments were performed according to Taguchi's design methodology. Four parameters were selected: temperature, suspended solid quantity, salinity, oil/water surface ratio. Each of these parameters was set at two levels in order to be represented by 8 different mixtures of oil and water. Water was allowed to disperse on the surface before starting a gentle magnetic stirring for 2h without vortex. The beaker was placed in a thermostatic chamber and darkness to avoid PAHs photodegradation. A large variation in composition of the water soluble fraction depending on oil type was highlighted. The chemical profile of the water soluble fraction was unlike that of the parent oil, but contained water-soluble compounds (naphthalene, anthracene) which constitute the major part of the water soluble fraction. For Heavy fuel and Jet, all parameters have been taken into account. For Bitumen, only suspended-soldid quantity can be neglected. For Domestic fuel, Gasoline, Diesel, salinity and suspended-solid quantity can be neglected. For all oils, the water soluble fraction was minimised with low temperature. For all oils except, Bitumen and Heavy fuel, the water soluble fraction was minimised with high surface ratio.

WE 158

Transteins in recent sediments of the Continental Shelf of Yucatan, Mexico

V.M. Ceja, E.C. Chan, L.V. Oceguera, G.B. Gold

Center of Research and Advanced Studies of IPN, Merida, Mexico

Oil spills covering a representative range of commercially available products were tested. Dynamic tests at laboratory scale were performed to study 41 polycyclic aromatic hydrocarbons (PAHs) and derivates, among them 16 EPA priority pollutants. The coupling of the stir bar sorptive extraction (SBSE) and high performance gas chromatography mass spectrometry (HPGC-MS) was used. Limit of quantification were lower than 1 ng/L for each compounds. Experiments were performed according to Taguchi's design methodology. Four parameters were selected: temperature, suspended solid quantity, salinity, oil/water surface ratio. Each of these parameters was set at two levels in order to be represented by 8 different mixtures of oil and water. Water was allowed to disperse on the surface before starting a gentle magnetic stirring for 2h without vortex. The beaker was placed in a thermostatic chamber and darkness to avoid PAHs photodegradation. A large variation in composition of the water soluble fraction depending on oil type was highlighted. The chemical profile of the water soluble fraction was unlike that of the parent oil, but contained water-soluble compounds (naphthalene, anthracene) which constitute the major part of the water soluble fraction. For Heavy fuel and Jet, all parameters have been taken into account. For Bitumen, only suspended-soldid quantity can be neglected. For Domestic fuel, Gasoline, Diesel, salinity and suspended-solid quantity can be neglected. For all oils, the water soluble fraction was minimised with low temperature. For all oils except, Bitumen and Heavy fuel, the water soluble fraction was minimised with high surface ratio.

WE 159

Toxicity of mixtures of petroleum products to the marine organism Mysidopsis juniae

C.Vaz, R.F.S. Bohm1, R.A. Gonçalves1, E.C. Spitzner1, T. Kleine1, C.E. Galoski1, M.G. Lobo1, W.G. Matias1, T.M.N. Oliveira1

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In Brazil, after the discovery of the Pre-salt layer, a big reservoir of oil that extends from the northern Campos Basin to the south of the Santos Basin, new technologies are being sought for the exploration and exploitation of oil. Questions about the level of preparation for the exploitation of reserves at great depth are uncertain as well as the level of preparation for such operations. Environmental impact studies such as the one performed in the Gulf of Mexico (USA) and the recent one in the Campos Basin (Brazil) in November 2011, raise the alertness of the gravity of high levels of the toxic oil and its products, especially diesel and gasoline. Gasoline consists of a wide range of components, and benzene, toluene, ethyl benzene, xylenes, as well as naphthalene and benzene are considered hazardous. This study explored the effects of various gasoline concentrations on the test organism Mysidopsis juniae. Three diesel samples, 100% of diesel in the first, 10% of diesel in the second and 5% of diesel in the third were used to study the toxicity. For the detection limit, negative correlations between barium and nickel and a positive correlation between the TMs were found. Barium exceeded in three samples the reference value proposed by the NOAA (AET of 48 ugg). In the case of the nickel found in samples and in the residual area of the samples, none of the reference values of the TEL and PEL were exceeded. Correlations between metals and hydrocarbons are showed.

WE 160

Acute toxicity and kinetics of diesel fuel using Mysidopsis juniae microcrustacean

C.Vaz, R.F.S. Bohm1, R.A. Gonçalves1, E.C. Spitzner1, T. Kleine1, C.E. Galoski1, M.G. Lobo1, W.G. Matias1, T.M.N. Oliveira1

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In Brazil, after the discovery of the Pre-salt layer, a big reservoir of oil that extends from the northern Campos Basin to the south of the Santos Basin, new technologies are being sought for the exploration and exploitation of oil. Questions about the level of preparation for the exploitation of reserves at great depth are uncertain as well as the level of preparation for such operations. Environmental impact studies such as the one performed in the Gulf of Mexico (USA) and the recent one in the Campos Basin (Brazil) in November 2011, raise the alertness of the gravity of high levels of the toxic oil and its products, especially diesel and gasoline. Gasoline consists of a wide range of components, and benzene, toluene, ethyl benzene, xylenes, as well as naphthalene and benzene are considered hazardous. This study explored the effects of various gasoline concentrations on the test organism Mysidopsis juniae. Three diesel samples, 100% of diesel in the first, 10% of diesel in the second and 5% of diesel in the third were used to study the toxicity. For the detection limit, negative correlations between barium and nickel and a positive correlation between the TMs were found. Barium exceeded in three samples the reference value proposed by the NOAA (AET of 48 ugg). In the case of the nickel found in samples and in the residual area of the samples, none of the reference values of the TEL and PEL were exceeded. Correlations between metals and hydrocarbons are showed.

WE 161

Acute and kinetic toxicity of diesel fuels from Colombia and Brazil

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Accidents resulting from leaks and oil spills have worried the industry, the government and the civil society. Of all the petroleum products, it is important to evaluate the effects of gasoline when it gets in contact with water. The monosazardic hydrocarbons present in gasoline such as benzene, toluene, isomers of xylene and ethyl benzene (aromatic hydrocarbons) are the most hazardous components. The aim of this study is to evaluate the acute toxicity and kinetics of the toxicity of gasoline. This study was conducted at the Laboratory of Environmental Toxicology UNIVILLE - in São Paulo, Brazil.
Francisco do Sul. In order to carry out this test a stock-solution was prepared with 50 mL of gasoline and 950 mL of deionized water, amounting to 1 liter. This mixture was stirred continuously for a period of 24 hours in order to separate the water-soluble portion. For the toxicity tests concentrations used were 0.1%, 0.5%, 1%, 1.5%, 2%, 4% and 10% (volume / volume) prepared in seawater reconstituted in 200 mL flasks. In order to set up the test, 5 Mydaspsis juniae between 3 to 5 days old were placed in each flask with the water. The experimental conditions were 32 for salinity, 12h light and 12h darkness for photoperiod and 25 °C for temperature. The organisms were fed once a day with Artemia sp. nauplii. The count was made every 2 hours to complete 12 hours and afterwards the count is made every 12 hours to complete 48 hours. Bioassays were performed in triplicate. The LC50 (48 h) calculated by probit method and was 1.97% (concentration of the soluble fraction). For the kinetics of toxicity was possible to observe effects of 24h on the 1 day. At concentrations below 2% the effects of the 24h of testing, At 4% concentration, the effects remained until the 48 hours, while at 10% concentration mortality of the population after 24 hours of test was 100%. The use of. M. juniae made it to be possible to evaluate the toxicity of a substance and it is necessary that the substance is handled with care to minimize environmental risks.

WE 162
Toxicity, fractionation, identification and potential estrogenic effects of acid extracts of oil sands process waters
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Conventionally rodenticides are controlled by their potential toxicity as such substances can pose serious environmental risks. However, the risks associated with the use of rodenticides can be further reduced by consideration of risk mitigation measures. Such measures can be implemented in two broad categories: 1) those associated with the use of the product and 2) those associated with the product itself. In the presentation I will discuss the latter, and the reasons for these measures. I will also hint at the wider implications associated with these strategies.

WE 163
Risk mitigation management for rodenticides
M.R. Maristella, Instituto superiore sanità, Roma, Italy

Risk mitigation management for rodenticides

1. Introduction
Rodenticides are regulated in Europe as biocides or as plant protection product regulated under Council while in North America are regulated under restrictive federal legislation and through generation AVKs, which normally PBT compounds, which would normally restrict their use: notwithstanding that, as rodenticides are not allowed to be used in the environment. The most appropriate risk mitigation measures are closely linked also to the design and pack size as well as composition and formulation type. The inclusion of a bittering agent, such as 4-nitrophenyl2-nitro-1,2-phenylenediamine, while inclusion of a blue dye prevents the product unachieved, the term ‘professional’ and 2. Associated to the approvals of these compounds can cause possible diverging interpretations: harmonized definition of “trained” at the moment fulfill different or no requirements among EU. Instead of using words as “domestic” or “amateur”, an unique definition of “non-professional” should be taken into account. Rodenticide products shall be labelled in accordance with preparations directly to be sufficient for the specific risks. Thus, in addition, all packaging of anticoagulant rodenticides need to show specific safety precautions in the form of additional harmonized standard phrases.

3 Conclusions
The safe use of rodenticides is highly dependent on the application, formulation and weight of sold baits, 2nd generation anticoagulant substances might have to be reviewed again after Annex I inclusion as candidates for comparative assessment. Specific phrases for special risks and safety precautions should be defined, in addition to good practice guidelines and training for professional users.

References

WE 164
Risk reduction in rodenticide use - does it work?
K.A.ハウラント
Swedish Chemicals Agency, Sundbyberg, Sweden

Rodenticides, in particular the second generation anticoagulants (SGARs), are very potent toxins. In the EU they are regulated as biocides by the Biodical Products Directive (BP, 98/8/EC) and also as plant protection products by the Directive 91/414/EEC (PPPD). There are today five active substances included in Annex I of the BPD, i.e. approved for use in biocidal products (brodifacoum, bromadiolone, difenacoum, dibutylstilbene disulfonate and flocumate) and two of those (bromadiolone, difenacoum) are included in Annex I of the PPPD. Their strong effect is similar on target organisms as well as non-target organisms and the environmental risk assessments of these compounds conclude that the risk for secondary non-target effects among wildlife is very high. Studies on wildlife have confirmed this and SGARs have been found in non-target animals such as rodents, predatory birds and mammals. The SGARs are approved only because there is a strong need to control rodents like rats and mice and because the existing alternatives are not sufficient to carry out this task.

Associated to the approvals of these compounds are a number of risk reduction measures, e.g. use of secured bait stations, use in limited time periods, recovery of dead animals and leftover bait and also the restriction to professionals only. The extent to which these measures shall be used is not specifically regulated and their efficacy is not allowed access. The authorised use could be limited for the outdoor use to professionals only, whilst the amateur use in a ready-to-use product may be restricted to indoor use. The most appropriate risk mitigation measures is closely linked also to the design and pack size as well as composition and formulation type. The inclusion of a bittering agent, such as 4-nitrophenyl2-nitro-1,2-phenylenediamine, while inclusion of a blue dye prevents the product unachieved, the term ‘professional’ and the linked subcategories can cause possible diverging interpretations: harmonized definition of “trained” at the moment fulfill different or no requirements among EU. Instead of using words as “domestic” or “amateur”, an unique definition of “non-professional” should be taken into account. Rodenticide products shall be labelled in accordance with preparations directly to be sufficient for the specific risks. Thus, in addition, all packaging of anticoagulant rodenticides need to show specific safety precautions in the form of additional harmonized standard phrases.

WE 165
Application of a human dimensions and landscape ecology framework to evaluate rodenticide use and human-wildlife interactions within ecosystems
R.C. Topping
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Research on Second Generation Anticoagulant Rodenticides (SGARs) often involves the toxicokinetics and -dynamics of products within organisms and observation of secondary non-target effects among wildlife. However, details about the human mechanisms and drivers for SGAR use, like product selection, use behaviors, target species, and interactions of human behaviors and characteristics of the broader landscape may influence human-wildlife conflict and human interactions with wildlife and nature within ecosystems. Human land use practices may contain attractive habitat characteristics that draw wildlife into proximity of residences, resulting in infestation and/or damage. Human reaction to wildlife presence, regardless of whether wildlife species are exotic or native, may result in behaviors that include rodenticide use both indoors and outdoors. Outdoor product use, or the result of animal consumption of products indoors followed by travel outside, can result in availability of raw or ingested products that may be consumed by wildlife. Humans also may seek to repair damage caused by perceived pests and in turn reinforce landscape characteristics attractive to wildlife. Therefore, application of a human dimensions and landscape framework not only allows for evaluation of human mechanisms of rodenticide use, but a greater understanding of human-wildlife interaction within landscapes. Such information can aid assisted-grow product manufacturers and policymakers in designing products and application procedures that both meet consumer needs for pest control and minimizing impact of products on non-target species.

WE 166
Secondary exposure of Red Kites (Milvus milvus) to anticoagulant rodenticides
J.M. Hughes, E.A. Sharp, M.J. Taylor
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Red Kites became extinct in Scotland in the late nineteenth century but a recent reintroduction programme has resulted in the re-establishment of a breeding population. Science and Advice for Scottish Agriculture (SASA) operates the Wildlife Incident Investigation Scheme (WIIIS) in Scotland and routinely monitors raptor carcasses for anticoagulant rodenticide residues. Residue testing of tissues samples from more than 100 red kites over the last decade has found that around 80% of individuals contained measurable rodenticides and that > 30% contained multiple residues. In addition, around 20% of the sample had total residue levels of > 0.2 mg/kg, a concentration that has been correlated with clinical evidence of rodenticide related mortality in several raptor species. In this sample, rodenticide exposure was attributed as the cause of death in
Ca 20% of individuals, not all of whom were confirmed by post mortem and the data included some individuals that may have been deliberately poisoned.

Although this is a limited dataset, patterns of red kite exposure were fairly consistent over time and were significantly greater than found in a range of other UK raptor species. Whilst, it has been hypothesised that the intensive monitoring involved in reintroduction programmes may increase the likelihood of carcass analysis and residue detection, the red kite’s lifestyle and feeding ecology make it particularly vulnerable to rodenticide poisoning. ASA also conduct biennial surveys of rodenticide use on arable farms and there is a correlation between the relative quantity of active ingredients applied and their detection rates in tissues. These data suggest that further quantification of exposure and associated risk of rodenticides to red kites is warranted and that possible deficiencies in working practice and compliance among rodenticide users in areas where red kites are resident may need to be addressed.

WE 167
Anticoagulant rodenticides levels in Eurasian eagle owls (Bubo Bubo) from Southeastern Spain
P. Gómez-Ramírez, E. Martínez-López, P. María-Mojica, A.J. García-Fernández
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Anticoagulant rodenticides (AR) have not been routinely included in biomonitoring studies. However, this type of toxicants may pose a risk for birds, especially those that feed on rodents. In addition AR have shown a high frequency of detection in bird samples from UK and USA, including in animals without haemorrhages. In contrast, data regarding rodenticides levels in Spanish wildlife is restricted to cases of suspected poisoning. In Southeastern Spain, the Eurasian eagle owl can live close to agricultural areas due to the abundance of small rodents. In order to evaluate ARs exposure from ARs in arable fields, live captured and dead or died at The Wildlife Rehabilitation Centre “Santa Faz” (Alicante). Also, blood samples from 9 adult Eagle Owl that were caught between 2008 and 2010 and 41 chicks born in the same period were analysed. Samples were extracted with acetonitrile to look for the 5 most frequently used AR in Spain and analysed with LC-MSMS. AR were detected in 83% of the sampled populations (26%), and AR were more abundant and frequent compounds, with 4% and 17% respectively. These most common compounds are the key-parameters of such regional systems. Based on a systems approach, this collaborative research network involves academic researchers, farmer, game, conservationist and may modulate prey/predator relationships; Duhamel et al. (2000) that networks of Talpa europaea, was slowed down by ploughing and cattle tramping and was modified by neighbouring landscape (10 - 100 ha). On a local scale (area of about 0.01 km2), Duhamel et al. (2000) that AR have been related to toxic effects in birds. The probable due to a shot, which caused its admission in the Rehabilitation Centre. Levels above 10 ng/g were found in two other ARs that died due to electrocution and truama. It should be recalled that the impact of AR must extend well beyond those cases in which acute lethal haemorrhage is the proximal cause of death. Sublethal haemorrhages may alter locomotion or cause lethargy, predisposing to birds, accidental trauma or reduced food intake. Because 72% of these individuals presented levels above 10 ng/g, which are related to subcutaneous haemorrhage, we could suggest that these compounds could be involved in the cause of death. Acknowledgements: Generalitat Valenciana for providing the samples. Supported by the Spanish Government (CGL2004-5959/BOS, CGL-2008-4318/BOS), Seneca Foundation (08758/P/PO0).

WE 168
Monitoring impacts of vertebrate pesticides in the UK: 1993 to 2007
A.P. Buckley, C. Pressure
University of Reading, Reading, United Kingdom
The Wildlife Incident Investigation Scheme (WIIS) is a pesticide post-registration monitoring scheme operated in the UK. Incidents in which pesticides are involved in non-target casualties are recorded, their circumstances investigated and, where possible, attributed to cause. This paper describes 116 WIIS incidents that occurred between 1993 and 2007 and involved non-target casualties caused by exposure to vertebrate pesticides, including the anticoagulant rodenticides used for rodent control.

WE 169
Possessing or exposing? Birds of prey and anticoagulant rodenticide residues from the Wildlife Incident Investigation Scheme in England
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On a local scale (area of about 0.01 km2), Delattre et al. (2006), Morilhat et al. (2007, 2008) showed that ARs are also of major importance for wildlife. In order to evaluate AR exposure, liver samples were obtained during necropsy from 18 Eagle owls that arrived dead or died at the Wildlife Rehabilitation Centre. These data suggest that these compounds could be involved in the cause of death. Acknowledgements: Generalitat Valenciana for providing the samples. Supported by the Spanish Government (CGL2004-5959/BOS, CGL-2008-4318/BOS), Seneca Foundation (08758/P/PO0).

WE 170
Residue use of the red fox (Vulpes vulpes), as revealed by stable isotopes, uncovers variation in exposure to anticoagulant rodenticides
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1NERC Centre for Ecology and Hydrology, Lancaster, United Kingdom
2University of Reading, Reading, United Kingdom
Anticoagulant rodenticides (ARs) are widely used to control commensal rodent species. However, their use can lead to the exposure of non-target species. Exposure varies widely between populations of predators but the reasons for the variation in occurrence is poorly understood. Diet is undoubtedly a contributing factor but few studies have shown a clear link between the occurrence of ARs and diet of a predator. We therefore use stable isotopes to infer resource use in a population of red foxes (Vulpes vulpes) in the Lincolnshire Wildlife Trust. Stable isotope signatures of prey and potential AR sources are collected from wildlife carcasses at 2 different sites. The isotope signatures are compiled into a dietary niche and compared to food web structures and rodent AR detection. The predicted diet is compared to the diet derived from AR sources and evidence of AR exposure is sought. If ARs are detected in harvested wildlife, their dietary use is compared to other populations from across the UK.

WE 171
Sustainable control of grassland small mammals: reducing the need for rodenticides and the risk for non-target species by a multi-scale approach
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Small mammals play a key role in the structure of many ecosystems. In France, a majority of the red foxes (Vulpes vulpes), the most abundant species of red foxes, the red foxes have been traditionally perceived as pests and targeted for control on a large scale despite their importance as key-species in their native ecosystem. This is also the case for the fossorial form of the water vole (Arvicola terrestris). This species can reach large population densities and causes heavy damage to grassland with subsequent economic losses for farmers (9,000 to 25,000 € for a 70 ha farm during an outbreak year in France). The presence of red foxes, however, higher population densities of A. terrestris help maintaining a large and rich community of voles predators. Such non-target species are impacted and also of Microtus arvalis, help maintaining a large and rich community of vole predators. In order to minimize the use of rodenticides in controlling A. terrestris populations, a long term research program has been undertaken since the late 80s, aiming at identifying the key parameters of such regional systems. Based on a systems approach, this collaborative research network involves academic researchers, farmer, game, conservationist, organizations and governmental and local administrations together.

On a regional scale (area of about 2500 km2), Delattre et al. (1999), Giraudoux et al. (1997), Fichet et al. (2000) provided evidence that M. arvalis and A. terrestris population dynamics were influenced by land use and land management practices.

On a sectorial scale (area of about 25 km2), Delattre et al. (1996), Delattre et al. (1999) showed that the landscape heterogeneity dampen population fluctuations and may modulate prey/predator relationships; Duhamel et al. (2000) that A. terrestris outbreak epicentres occur in homogenous grassland and Foltete et al. (2008) that hedgerows may slow down the propagation of outbreaks.

On a local scale (area of about 0.01 km2), Delattre et al. (2006), Moriilhat et al. (2007) showed that A. terrestris population growth was enhanced by larger galaxy networks of Talpa europaea, was slowed down by ploughing and cattle trampling and was modified by neighbouring landscape (10 - 100 ha). This led to new recommendations, including a more sustainable control of A. terrestris (Delattre and Giraudoux 2009) with substantial decrease in rodenticide utilization and lesser impact on non-target species.

WE 172
Determining critical liver concentrations of second generation anticoagulant rodenticides in Canadian predatory bird species
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2NRC Centre for Ecology and Hydrology, Lancaster, United Kingdom
3Université de Montréal, Saint-hyacinthe, Canada
Anticoagulant rodenticides (ARs) are widely used to control commensal rodent species. However their use can lead to the exposure of non-target predatory species. Exposure has shown a clear link between the occurrence of ARs and diet of a predator. We therefore use stable isotopes to infer resource use in a population of red foxes (Vulpes vulpes) in the Lincolnshire Wildlife Trust. Stable isotope signatures of prey and potential AR sources are collected from wildlife carcasses at 2 different sites. The isotope signatures are compiled into a dietary niche and compared to food web structures and rodent AR detection. The predicted diet is compared to the diet derived from AR sources and evidence of AR exposure is sought. If ARs are detected in harvested wildlife, their dietary use is compared to other populations from across the UK.

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Second-generation anticoagulant rodenticides (SGARs) are widely used to control rodent pests but exposure and poisonings occur in non-target species, such as birds of prey. Liver residues are often analyzed to detect exposure in birds found dead but their occurrence is now so widespread that their use to assess the toxicity of SGARs is problematic. We analyzed published data on hepatic rodenticide residues and associated symptoms of anticoagulant poisoning from 270 birds of prey using logistic regression to estimate the probability of chlorophacinone poisoning from different liver SGAR residues. We also evaluated exposure to SGARs on a national level in Canada by analyzing 196 livers from great horned owls (Bubo virginianus) and red-tailed hawks (Buteo jamaicensis) found dead at locations across the country. Calculated probability curves suggest significant species differences in sensitivity to SGARs and significant likelihood of toxicity much below previously suggested concentrations of concern. For example Great horned owls typically have PT values at least 10 times below the proposed thresholds of 0.1 - 0.2 mg/kg (Newton et al in litt.) or the 0.7 mg/kg proposed by the Rodenticide Registrants Task Force. If the latter value was accepted as a threshold, we estimate this would place 15 to 20% of all raptorial species at risk of acute toxicosis. Of the two species for which we had samples from across Canada, great horned owls are exposed to SGARs to a greater extent than red-tailed hawks and liver residue levels were also higher. Using our probability estimates of effect, we estimate that a minimum of 11% of the sampled great horned owl population was at risk of being directly killed by SGARs. It is likely the risk is now higher because most of the samples underpinning this analysis were from the 1990s. This is the first time the potential mortality impact of SGARs on a raptor population has been estimated.
Silver nanoparticles (AgNPs) are currently being very widely used in industry, mainly because of their anti-bacterial properties, with applications in many areas (1). Once released into the environment, the mobility, bioavailability, and toxicity of AgNPs in any ecosystem are dominated by colloidal stability (2). AgNPs released in wastewater may be converted into Ag+ ions, complexed with ligands, agglomerated or still be present as nanoparticles (3). By using HR-STEM and electron energy-loss spectroscopy (EELS) we have assessed the surface changes of citrate-stabilized AgNPs prepared in the lab after being exposed to different environments, using fulvic acid solutions similar to fresh and seawater and the presence of light. Preliminary results show that particles in fulvic acid remain stable and unchanged for months, while particles exposed to light change in size and form.

References:
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WE 178 Behaviour of Ag NPs in the standardized ecotoxicity test media and natural waters
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3Chapman University, Chapman University, California, United States of America

To understand the risks of Ag NPs to aquatic organisms, many researchers have performed toxicity tests for NPs using standardised ecotoxicity test media. However, the specification of NPs in these standardised media can be very different from the specification that occurs in the natural environment. Results from standardised ecotoxicity exposure studies therefore have little relevance to toxicity in the natural environment. Therefore, in this study, the aggregation of negative charged Ag NPs (average size: 30nm) was investigated over 48h in a range of standardised media, namely: artificial pond water (APW), OECD algal and daphnia M4 media, Gamberg’s B-5 basal medium, artificial salt water (ASW) and US EW test media covering a range of hardnesses. The effect of the presence of a test organism on aggregation was also assessed with following combinations: M4/Daphnia magna/48h, APW/Gammarus pulex/96h, and Gamberg’s media/Leuca mini/24h. To explore the effects of natural organic matter on the aggregation of Ag NPs, tests were also done in the presence of humic acids (HAs) at concentrations of 1mg/L and 5mg/L of HAs. Aggregation was also assessed in de-ionised water. To assess behaviour in the natural environment, studies were done on 23 natural water samples which ranged in pH from 3.79-7.90. The aggregation of Ag NPs, in all tests was followed using nanoparticle tracking analysis using a NanoSight2 (NanoSight Ltd, Amesbury, UK). Two-Way analysis of variance (PASW 18; SPSS Inc.) was used to evaluate differences, the significance level was p<0.05.

No aggregation was seen in de-ionised water and EPA soft water. In the other media the NPs were found to aggregate very quickly and the size of the aggregates had reached a maximum within 6h. For the Lemma and algae media, the particle size reached a maximum after 2h and 6h respectively after which time, the particle size decreased. Greatest aggregation was seen in the APW and the EPA hard water. Addition of the HA reduced the particle size. The addition of D. magna to M4 media and L. minor to Gamberg’s media had no significant effect on mean particle size. The addition of G. pulex to APW significantly reduced the mean particle size of the Au NPs over 96h. Of the 23 natural water samples, 19 samples had a pH value between 3.79-7.90. In these samples, no aggregation of the Au NPs was observed (≈ 50nm). Four samples showed significant aggregation (+ 100nm) at pH value between 7.68 and 7.90.

WE 179 Weathering of polycarbonate composites containing 14C radio-labelled carbon nanotubes
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Due to their outstanding mechanical, electrical and thermal material properties, carbon nanotubes (CNT) are among the most intensively studied nanoscale materials. Composing with organic or inorganic materials, CNTs can be fabricated into transistors, sensors and other electronic devices. These properties have resulted in the end of the lifecycle of a polymer composite containing radioactive (14C)-labelled multi-walled CNT (MWCNT) to trace their fate due to weathering. The 14C label allows the determination of possible CNT release after disposal of composites in the environment.

Here, 14C-MWCNT containing polycarbonate composites were prepared by dispersing the CNT in chloroform by ultrasonication and, afterwards, mixing with solutions of polycarbonate. Solution-cast thin films were prepared and characterized. Accelerated weathering of the thin films was studied by irradiation with a sun-like Xe-arc lamp under standard conditions. The irradiation dose was varied to study the time scale of the polymer decomposition. Released debris was collected and quantified with respect to composition and amount. 14C-MWCNT content in order to estimate what fraction of CNTs may possibly be released upon environmental disposal of CNT-containing polymers. The weathering is analyzed by X-ray photoelectron spectroscopy, X-ray electron microscopy, infrared absorption spectroscopy, X-ray photoelectron spectroscopy and Raman spectroscopy.

WE 180 Environmental exposure modeling of engineered nanomaterials and comparison to their corresponding bulk/total material flows
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There is a need to assess the environmental risks of engineered nanomaterials (ENMs) due to their rapidly increasing application in consumer products, which might lead to environmental exposure to these ENMs. Using probabilistic material flow modeling, we have reported in 2009 the first environmental concentra-tions for different ENMs in various environmental compartments. The present article aims to prepare up-to-date and comprehensive concentration estimates of ENMs by considering both ENMs and also their corresponding bulk/total material flows (e.g. pigment-TiO2, silver and zinc metal and carbon black). The analysis is conducted in two stages: (1) prediction of updated environmental concentrations of five ENMs, namely nano-TiO2, nano-ZnO, nano-Ag, Carbon Nanotubes (CNT) and Fullerene (C60); (2) prediction of the environmental concentration of the nano fraction of bulk TiO2, a similar probabilistic material flow modeling is performed.

The results of the prediction of environmental concentration (PEC) for the five ENMs and different environmental compartments (wastewater, surface water, sewage sludge, sediments) are compared to the previous data reported in 2009 (Sun et al., 2009). These comparisons with 2009 enable a more realistic assessment of the risks of ENM to the environment by focusing not only on the “nano” issue but taking a more complete view and relating the ENM to already existing compounds.

WE 181 Characterization and quantification methods for assessing the interaction of silver nanoparticles with different fresh and aquatic media
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The industrial expansion of manufactured silver nanoparticles (nAg) production will likely enhance their accumulation into environmental and biological matrices. nAg may be found in a consumer products, applying its biocidal potential. The free form Silver (Ag+), is a highly reactive and persistent entity, Release of Ag+ from nAg will depend on both solution and nanoparticles composition. The main goal is to develop methodologies to characterize and quantify nAg and Ag+, in different aquatic growth culture media: algae (Woods Hole MBL), daphnia (ASTM hardwater) and cell-lines (D-MEM). FFF (Flow field fractionation), using online UV, ICP-AES and LIFES (Laser-induced plasma atomic emission spectroscopy) detection, provides separation and characterization of nAg. More conventional approaches, as equilibrium dialysis (ED), microfiltration and ultrafiltration were used to quantify the total dissolved Ag+ using ICP-AES. Mixtures of citrate-stabilized nAg (40 & 100 nm) and AgN3 were added to culture media (50 ppb). Two timepoints (T0 & Tend) were used, following the respective OECD guideline of each culture organisms. Along the time (days), Daphnia tesita media a higher amount of Ag was accumulated. In ASTM with AgN3 (1 KDa), the external solution decreased from 49 ppb to 3.7 ppb, while the final Ag value in the cell ed was 11 ppb. Similarly values were found in MBL and D-MEM, since acid extraction of the test containers showed substantial adsorption. The hypothesis is that the ED process is slower than absorption to the solution containers. Therefore, the final value in the dialysis bag represents equilibrium. Dispersion of AgN3 in the culture media shows changes in the fractogram owing to aggregation or NP-membrane interactions in the FFF On-line ICP-AES was used for determination of mass distribution. The comparison among all the quantification methods and all the characterization detectors and their implications on the results will be discussed.

WE 182 Development of a new method to extract and analyze fullerenes and fullerene-derivatives from soil using HPLC with UV and fluorescence
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SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
Fullerenes are carbon-based nanoparticles considered as some of the most promising materials in emerging nanotechnology. Their current use varies from industrial to household applications and the expected increase in production is likely to imply a release of such materials into the environment. In particular, fullerenes have been suggested to enter the terrestrial ecosystem by direct release (e.g. leaching from fullerenes-containing materials) as well as from the atmosphere and transport through aquatic or terrestrial pathways. Their environmental behavior is largely unknown and standard procedures for their analysis in soil are missing.

In the present study we investigated the extraction and analysis of several fullerenes (C60, C70) and exohedral-functionalized fullerene derivatives ([60]PCBM, [60]PCBB, [60]PCBM, [60]TECBM, bis-[60]PCBM and [70]PCBM) from soil matrices. For the first time such a large variety of fullerenes dissolved in toluene and water were chromatographically and spectroscopically characterized. For fullerenes detection and quantification several methods of extraction (AESE, sonication, shaking in different conditions) were tested and compared for their efficiency in terms of recovery. UV spectroscopy is a powerful tool to detect and quantify fullerenes owing to its high linearity and low LoD. We were able to analyze soils down to fullerene contents of 1·µg·kg⁻¹. The lowest level of quantification could be even decreased to 0.1·µg·kg⁻¹ using mass spectrometry for detection. Our suggested extraction method for soils has to be further tested with soils differing in important properties controlling the fate of fullerenes, such as the contents of organics, C, Cl and Fe oxides. Furthermore, our approach to dissolve fullerenes in water will give studies on the environmental behavior of fullerenes a large boost.

WE 183
How to evaluate the exposure to engineered nanoparticles in freshwater?
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The increase in production and use of engineered metal-oxide nanoparticles makes the exposure to nanoparticles (NPs) more likely in the natural environment. Current state of knowledge on fate, exposure and toxic effects of NPs makes it difficult to evaluate the environmental risk posed by the NPs. Several reports on nanotechnology underline the importance to further progress in research on fate and transport of NPs in the environment, as this field of research is still rather undeveloped. Therefore, an actual and current evaluation of the predicted environmental concentration (PEC) of NPs is mainly based on material flow analysis (MFA) where the NPs are treated as bulk material. In such a scenario the particular mate of the material is not considered. A correct evaluation of the exposure, in principle needs to consider all fate processes in order to estimate the bioavailable fraction. Two processes seem to be significant are the fate processes of aggregation (incl. sedimentation) and dissolution. Environmental scientists have recently encouraged modeling of NP fate in freshwater based on colloidal chemistry and it has also been recognized that abiotic factors such as ionic strength and pH could influence the colloidal behavior of NPs. Therefore, based on colloidal and water chemistry, the aim of this research is to develop a fate model of water evaluating the suspended concentration of metal-oxide NPs (e.g. TiO2) to which organisms are exposed. The framework is focused on two main processes that affect the environmental behavior of the metal-oxide NPs in freshwater: aggregation (incl. sedimentation) and dissolution. To model the agglomeration and dissolution in freshwater of spherical NPs are assumed along with Brownian motion, fast aggregation, and the correlation of effects to the suspended NPs. The considered freshwater compartment is a box with a constant inflow of NPs where water temperature and fluid viscosity are constant. The framework combines colloidal science and water chemistry and takes into account abiotic factors that influence the dissolution and aggregation, which leads to gravitational sedimentation. The developed model will in the end be applied in Life Cycle Impact Assessment (LCIA) with the aim to improve the substance coverage of the LCIA method.

WE 184
Impact, behavior and fate of silver nanoparticles in aquatic environments
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Engineered nanoparticles and materials are increasingly used in a broad variety of consumer products; their economic importance has risen strongly over the last years. Especially silver nanoparticles (Ag NP) are frequently added to products such as sportswear and cosmetics nowadays. In contrast, knowledge about characteristics and effects of released Ag NP in the environment is still limited.

The German Federal Ministry of Education and Research (BMBF) has launched the “HighTech-Strategic” programme “NanoNature” in 2008 to support and promote research projects aiming to understand the impacts of engineered nanoparticles on the environment in general and on organisms in particular. The BMBF-funded project “ENPVIS” (ENgineering, PRecision, Prediction and Sizing) focuses on the environmental fate of Ag NPs.

The Ecotoxicological Laboratory of the German Federal Environment Agency (UBA) as one of 17 “UMSICHT”-project partners is currently investigating the biological effects of Ag NP on aquatic organisms. Main objective is to provide a solid data set on Ag NP in aquatic ecosystems which can be used for hazard estimation and risk assessment in chemicals policy.

Therefore, the effects of Ag NP has been studied in several species representing different trophic levels in aquatic ecosystems, ranging from luminous bacteria (Vibrio fisheri) and cyanobacteria (Anabaena flov-aquea) to green algae (Desmodesmus subspicatus) and higher macrophytes (Lemna minor), invertebrates (Daphnia magna) and vertebrates (fish species). All tests were conducted according OECD guidelines with draft protocols on ecotoxicological testing. In addition it should be demonstrated if, and where and in what quantity Ag NP is incorporated by the organisms over time. All results regarding Ag NP were compared to the effects of silver nitrate to distinguish the impact of Ag NP and silver ions.

Furthermore, the effects of Ag NP have been accompanied by several analytical methods such as dynamic light scattering and UV/VIS spectroscopy to describe the particle size distribution of Ag NP, optical microscopy to visualize Ag NP accumulation and incorporation and determination of Zeta potentials and silver contents (total silver and silver ions) in aqueous solutions to characterize Ag NP and its effects on aquatic organisms.

WE 185
Effect of copper ion on adsorption of chlorinated phenols and 1-naphthylamine to surface-modified carbon nanotubes
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Understanding adsorptive interactions between organic contaminants and carbon nanotubes is critical to both the environmental application of carbon nanotubes as special adsorbents and the potential impact of the transported contaminants on the fate and transport of organic contaminants in the environment.

To understand how transitional metal ions with strong complexing capabilities might affect the adsorption of hydroxyl- and amino-substituted aromatics to different functional groups of carbon nanotubes, the adsorption of organic compounds (1,2-dichlorobenzene, 2,4-dichlorophenol, pentachlorophenol, and 1-naphthylamine) to different functionalized carbon nanotubes (functionalization-rich SWNTs and N-functionalization-rich SWNTs) was evaluated.

Adsorption of 2,4-dichlorophenol, pentachlorophenol, and 1-naphthylamine to O-SWNT and N-SWNT can be significantly enhanced in the presence of Cu(II) (50mg/L), especially silver nanoparticles (Ag NP) are frequently added to products such as sportswear and cosmetics nowadays. In contrast, knowledge about characteristics and effects of released Ag NP in the environment is still limited.

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The large-scale use of titanium dioxide nanoparticles (n-TiO2) in consumer and industrial applications suggests potentially significant releases into the environment, raising environmental health and safety concerns. Potentially impacted ecosystems include estuarine and coastal biota/organisms. Previous results from ecotoxicological studies with n-TiO2 dispersed in salt exposure media are difficult to interpret due to fast flocculation and sedimentation phenomena, affecting the concentration of suspended material. The goal of this study was to investigate the effect of agglomeration, a relevant natural polyvalency biopolymer, in stabilising n-TiO2 in artificial seawater solutions usually applied in ecotoxicity bioassays. The size distribution of n-TiO2 dispersions was monitored by dynamic light scattering (DLS) measurements, while the sedimentation behaviour was investigated with a stability analyser under centrifugal forces. Under these conditions n-TiO2 agglomerates were formed with average sizes < 270 nm for the entire period of observation, as measured by DLS. The size distribution remained constant after re-suspension, indicating that no agglomeration occurred after deposition. Preliminary ecotoxicity tests on two marine species, the crustacean Artemia franciscana and the micro-alga Phaeodactylum tricornutum, showed moreover that the presence of agglomate in the exposure media did not negatively affect the tested organisms.

WE 188
Dissolution, aggregation and transport of ZnO nanoparticles in aqueous matrices containing dissolved organic matter
Universitat de Lleida, Lleida, Spain

The fate and toxicity of engineered nanoparticles (NPs) in natural waters are strongly related to their physicochemical behaviour. In particular, aggregation and sedimentation modes of the transport of NPs towards organisms. In a specific case of ZnO, one must also take into account the important dissolution process, which is highly dependent on pH. Some authors even attribute the toxicity of ZnO NPs to the ensuing relatively high free Zn(II) concentrations, rather than to the NP form. Apart from pH and salinity, dissolved organic matter (DOM) plays a key role in the stability of NP suspensions, because adsorption of organic matter, such as humic acids or fulvics acids, onto the NP surface can dramatically vary its surface charge.

The recent technique AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) is specially suited for studying ZnO dissolve, given that it provides a direct and robust technique to contribute the knowledge of the properties of ZnO NP dispersions, which are essential for environmental impact and toxicity assessment. The determination of humic acid (HA) with the NPs that are analysed by UV-vis absorption and Laser Doppler Electrophoresis. It is shown that DOM adsorbs readily on the NPs and inhibits further aggregation by stabilizing the agglomerates due to electrostatic repulsion. The sedimentation of the HA-coated NP aggregates was studied by UV-vis spectroscopy and Dynamic Light Scattering. The results compared satisfactorily with a transport model based on Brownian diffusion and Stokes sedimentation of fractal, polydisperse NPs, which allows the estimation of their effective density. The implications in the context of environmental fate of NPs and uptake fluxes for in vitro toxicity experiments are also addressed.

This research has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 229244 (ENNSATOX).

WE 190
Engineered nanoparticle disposition and agglomeration in artificial biofluids
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It is known that different environmental matrices (e.g., salt water, freshwater with humic acids, soils) cause changes in nanoparticle (NP) agglomeration states. These environmental matrix characteristics include biological fluids (e.g., salts, surfactants), so it is possible that similar disposition and agglomeration states will be seen within different biological fluids. The movement of NPs through biological matrices (e.g., gastrointestinal tract, respiratory tract) will expose NPs to different environments, which will likely alter the agglomeration state of the NPs. In this project, we examined the dispersion of 1 mg/ml coated silver (Ag) NPs in three artificial biological fluids (80% Ag, 20% blood proteins, and interstitial, to simulate what happens in the stomach and lungs, respectively). Ethyleneaminoethanethiosuccinic acid (EDTA)-coated Ag NP (80 nm) settling in gastric fluid was measured spectrophotometrically over a 30 minute period. Lower pH (e.g., 1–3) caused EDTA-Ag to settle quicker, whereas EDTA-Ag stayed more dispersed at higher pHs (e.g., 5–7). In aeroalveolar fluid, both 20 and 80 nm citrate-Ag agglomerated, as determined by dynamic light scattering (DLS) analysis and field flow fractionation. Dissolved plasma proteins slowed little settle Ag and increased Ag NP size significantly. In both aeroalveolar fluid and plasma, Ag NPs remained dispersed in citrate-Ag with a diameter of 8 nm. No difference in Ag NP settling was observed between citrate-Ag and EDTA-Ag in plasma protein, citrate-Ag showed a decrease in Ag NP size over the 30 minute exposure. The size of Ag NPs in plasma protein did not change over the 30 minute exposure. The size of Ag NPs in plasma protein did not change over the 30 minute exposure.

NP coating and surface can dramatically vary its surface charge.

NP coating and surface can dramatically vary its surface charge.

The centrifugal force of 20 rpm was demonstrated how artificial biofluids can be used to predict the agglomeration of NPs within the body, giving researchers clues as to NP behavior within different organ compartments and chemical fractions the body may actually be exposed to (e.g., dissolved ions, nano-sized particles, micron-sized particles). These data may help researchers predict the actual amount of NP that is bioavailable, based on the NP dissolution rate and agglomeration size.

WE 191
Mechanism of nanoparticle interference in biochemical assays
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Nanomaterials are becoming common in commercial products and the assessment of their safety is crucial. The evaluation of the toxicity of nanomaterials can be hindered by conflicting reports demonstrating differential degrees of toxicity with the same nanoparticles. We have shown that nanoparticles can interfere with biochemical assays commonly used to assess physiological and biochemical status of an organism. We show that protein assays, the catalase assay, alamar blue and MTS assays, and LDH assays are all sensitive to the presence of Ag NPs in cell culture in all the mechanisms by which nanoparticles can cause cytotoxicity: increased uptake, binding proteins, and changes in intracellular redox. We have shown that citrate-Ag (20 or 80 nm) incubation with cultured cells for 24 hours causes increased cell death in a well-vascularized carbon nanoparticle, and single walled carbon nanotubes. We predicted that nanoparticles could affect assays by binding to proteins and dyes present in the assays, affect structure and activity of these molecules, change the fluorescence or absorbance characteristics of the molecules, and/or compete for fluorophore and reagent. We have shown that nanoparticles could bind and change the structure and agglomeration state of the dyes (e.g. tetrazolium-based dyes, resorufin) and proteins (e.g. bovine serum albumin). Spectrophotometric measurements were used to establish the change in optical properties after incubation with nanoparticles. The ability of nanoparticles to reduce dyes was examined using X-ray photoelectron spectroscopy. This work will contribute to the understanding of the unique physicochemical effects of nanomaterials, and help nanotoxicologists control for nanoparticle interference in their biological assays, which could reduce conflicting toxicity reports.

WE 192
Quantification of a series of fullerenes nC60 and related transformation products in water
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This study describes the development of a sensitive analytical method to quantify nC60 in water, using accurate mass screening liquid chromatography-hybrid linear ion trap Orbitrap mass spectrometry. The formation of oxidation, reduction and photochemical transformation products of nC60 was studied as well. The developed method enabled to detect and quantify aqueous concentrations of the summed nC60 and its transformation products as low as 5 ng/L. It was observed that nC60 could be oxidized to C60oxygen, but that oxC60 was not present in measurable quantities of the parent C60. Despite the high sensitivity of the developed method, no nC50 or transformation products were detected in an array of Dutch surface waters.

HPLC/UV/MS analysis was performed with an LC-DAD-LTQ-Orbitrap MS system. Both ESI and APCI were used for ionization. Aqueous samples were extracted using preconditioned C18 SPE columns, surmounted by filtration columns. Surface water samples were collected throughout the Netherlands.

The tolueño/methanol eluens and ESI ionization method resulted in most sensitive determination of C60 with liquid chromatography-hybrid linear ion trap Orbitrap mass spectrometry.
Currently, this method of analysis is being adapted and applied to 13 different derivatives of fullerene. Results of this further study will be presented at the conference.

The deposition of silver onto other regions in Switzerland, the deposition rates were slightly higher, but in the same order of magnitude. The same holds for the silver concentrations in the mosses.

Silver concentrations in the mosses were between 0.002 and 0.033 µg g\(^{-1}\) have the potential to cause cell membrane damage and may enter cells.

Effects of ENP, which have been found for different organisms, ENP are suspected to influence the transport, bioavailability and toxicity of a range of environmental organisms and biological systems. Silver nanoparticles are used in a number of consumer products due to their antimicrobial properties, but on entering aquatic systems (e.g., via wastewater treatment plant outflow pipes) they have the potential to disrupt a set of microbial communities and their processes. The aim of this study was to physically and chemically characterize capped and uncapped silver nanoparticles, measure their toxicity to pure bacterial cultures, and investigate their effects on key aquatic microbial processes including nitrification and hydrocarbon biodegradation.

Nano-particle characterization revealed that uncapped nanoparticles were larger in size than capped nanoparticles, but both types of nanoparticles formed aggregates in natural estuarine water and seawater. Nanoparticle toxicity as measured by bacterial growth assays at three concentrations (0.5, 5 and 50 mg L\(^{-1}\)) revealed that capped silver nanoparticles completely inhibited the growth of Escherichia coli and Bacillus subtilis at 50 mg L\(^{-1}\) whereas uncapped silver nanoparticles caused an extension in lag phase in both bacteria at 50 mg L\(^{-1}\) but did not completely inhibit growth at any concentration. TEM images of NP-bacterial cell interactions indicate that AgNPs and Ag⁺ enter bacterial cells membrane damage and may enter cells.

In freshwater sediments amended with crude oil in order to stimulate microbially induced biodegradation, significant changes in microbial community structure were observed in the presence of 0.5 and 50 mg L\(^{-1}\) capped and uncapped nanosilver, yet hydrocarbon degradation rates were unaffected by their presence. In freshwater sediments amended with crude oil, the addition of AgNPs to stimulate nitrification, ammonium oxidation rates were significantly reduced after 1 day, 7 days and 14 days in the presence of 50 mg L\(^{-1}\) capped nanosilver, yet in estuarine sediments that underwent the same treatment, reduced ammonium oxidation rates caused by capped nanosilver (at 50 mg L\(^{-1}\)) began to recover after 14 days.

Results suggest that although nanosilver particles may have a detrimental effect on aquatic microbial communities and their processes, this will be dependent on the physical and chemical characteristics of the nanoparticle itself as well as the concentration it is present in.
was investigated using a novel mass balance based single-equilibrium approach. Only citrate coated gold ENP (AuNPCIT) were found to bind phenanthrene, with ENP adsorption and increase of FAs concentration are expected to promote not only the NPs stabilization but also the dissolution of NPs aggregates. NPs charge overcompensation is systematically achieved at high FAs concentration. In addition, our study suggest that very low concentrations of Fulvic acids > 0.05mg-L^-1 are sufficient to rapidly stabilize iron hydroxide NPs solutions at concentration < 5mg-L^-1, independently of the pH of the solution, and play important roles in the fast dispersion of NPs aggregates.

WE 198
Stability of hematite nanoparticles in function of pH and Fulvic Acids concentration

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Fulvic Acids (FAs) are playing important roles in the reactivity and transport of pollutants in aquatic systems. They are also suspected to play important roles in the surface chemistry of metal NPs and aggregation of nanoparticles. The exact conditions under which aggregation or dispersion occurs will depend on the nanoparticle surface charge properties, FAs concentration as well on the solution conditions, such as pH and ionic strength. The systematic study of stability-aggregation phase diagrams is therefore a key aspect in the prediction of the environmental fate and behavior of released nanoparticles in aquatic systems. In this study, we focused on investigating both the aggregation and aggregation of iron hydroxide nanoparticles (NPs) in the resulting surface charge transformations. By adjusting the pH, different nanoparticle surface charge scenarios are investigated corresponding to positively, neutral and negatively charged nanoparticle solutions. For each situation, the aggregation of negatively charged FAs at variable concentrations is considered by analyzing surface charge modifications and calculating the kinetic aggregation rates. Our results show that the nanoparticle aggregation rate is promoted only when the nanoparticle positive surface charge (solution pH less than the charge neutralization point) is compensated by the adsorption of FAs. In all the other cases the aggregation of FAs was faster than the aggregation of nanoparticles.

WE 199
Past and present dispersion state strongly affects the sorption behavior of carbon nanotubes

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Polydispersion = 0.3; crystalline phase: mostly anatase) are manufactured by laser pyrolysis at the ENEA Research Centre of Frascati. The exposure mode has been evaluated focusing on: the treatments of the sample, the volume of sample tested (50ml and 2ml) and the length of exposure (until 96h). Two experiment set-up have been performed. In the first one four treatment of the sample were performed, and the organisms were exposed to 50ml of substance. In the second experiment set-up the D.magna was exposed.

WE 200
The influence of exposure mode in the hazard identification of metallic nanoparticles: the case of TiO2 and Daphnia magna

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RESULTS indicated that dispersion can significantly affect the sorption behaviour of CNTs. On the one hand, sonication broke down CNT aggregates and greatly increased pyrene sorption (sorption increased by 1-10 orders of magnitude). Sorption surfaces newly exposed during sonication remained available to pyrene molecules even if aggregation occurred, supporting an irreversible effect of sonication. On the other hand, the presence of HA decreased the sorption of pyrene. At the highest HA concentration investigated (200 mg-L^-1), sorption was still 1.6 orders of magnitude stronger than sorption of pyrene to HA itself. This indicates that specific interactions between pyrene and HA are still occurring, in spite of a HA coating of the CNTs surface. A greater suppression of sorption by HA occurred when combined with a sonication pre-treatment. Sorption isotherm fitting indicated that the maximum sorption capacity, affinity and heterogeneity of CNTs surface were all affected by sonication and the presence of HA.

WE 201
Biotransformation of carbon-based nanomaterials by Horseradish Peroxidase

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RESULTS from ecotoxicology studies performed with nanoparticles (NPs) highlight the need for a consensus on how to administer NPs to aquatic organism in ecotoxicity tests. Recent findings suggest that ecotoxicity values depend on the method of treatments of NPs in solution and the setup of the experiment. With the aim of developing a standardized ecotoxicological testing of n-TiO2 depends on exposure mode. A suite of acute toxicity tests on Daphnia magna, extending the exposure length to 96h, with n-TiO2 were performed (OECD 202). The n-TiO2 (XRD size: 15 nm; DLS size: Zave = 183 ± 7, Polydispersion > 0.3; crystalline phase: mostly anatase) are manufactured by laser pyrolysis at the ENEA Research Centre of Frascati. The exposure mode has been evaluated focusing on: the treatments of the sample, the volume of sample tested (50ml and 2ml) and the length of exposure (until 96h). Two experiment set-up have been performed. In the first one four treatment of the sample were performed, and the organisms were exposed to 50ml of substance. In the second experiment set-up the D.magna was exposed.

SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting

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Nanoparticles (NPs) display special chemical properties because of their size, shape, composition and electronic structure. These properties lend NPs their functionality, but also present a potential risk for humans and the environment due to their small size and surface area. In aquatic environments, the presence of NPs can affect the health of waterfowl and the broader ecosystem.

**WE 203**
Effects of gold nanoparticles on green algae Pseudokirchneriella subcapitata and Chlamydomonas reinhardtii

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Gold nanoparticles are used in various fields, especially biological areas such as biosensor, carrier for drug and gene, and anti-cancer agent. We assessed the toxicity of gold nanoparticles (AuNPs; 10 nm) to green algae Pseudokirchneriella subcapitata and Chlamydomonas reinhardtii. They are widely present in freshwater ecosystems. Both test species were exposed to AuNPs (100 µg L⁻¹ and 1000 µg L⁻¹) in OECD algal media, and experiments were conducted for 72 hours. The growth rate of Pseudokirchneriella subcapitata was inhibited to 0.5 mg/L of AuNPs. Chlamydomonas reinhardtii was significantly influenced by 10 mg/L of AuNPs. Pseudokirchneriella subcapitata was more sensitive than Chlamydomonas reinhardtii. We observed that gold nanoparticles negatively affect the freshwater green algae. This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2011-0035895).

**WE 204**
Effects of aged Carbo-Iron composite nanoparticles on green algae and zebrafish embryos in combination with the environmental contaminant tetrachloroethylen

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Composite particles of colloidal activated carbon and zero-valent iron are under development to be used as in situ reagent for remediating contaminated groundwater (Carbo-Iron®). Activated carbon acts as a sorbent to bind contaminants such as e.g. tetrachloroethylene (PCE) and the iron in the activated carbon structure subsequently dechlorinates the contaminants while it is oxidized itself. Carbo-Iron particles are designed to remain in the environment after application in the aged form (iron oxide/hydroxide on activated carbon). Therefore, we accompanied the particle development with ecotoxicological studies to identify the hazardous potential of the catalyst after the reaction towards the aquatic model organisms Scenedesmus vacuolatus (green algae) and Danio rerio (zebrafish). We found that aged Carbo-Iron particles (that are no reducing agents anymore) are not toxic to zebrafish embryos up to 100 µg/ml upon 48 h of exposure. Furthermore, we found growth inhibition in the green algae Scenedesmus vacuolatus which we could show to be due to a shading effect. That means that toxicity is due to light absorption which is similar when algae are exposed to decreasing light intensities without particles.

PCE is an often occurring volatile groundwater contaminant exerting toxicity in many organisms. A combination of aged Carbo-Iron particles with PCE reduced its toxicity in zebrafish embryos assumed to be due to adsorption of PCE to the activated carbon and therewith a reduced bioavailability. In algae we expect a joint effect resulting from PCE adsorption as a response to light absorption. The Carbo-Iron particles themselves are not toxic to algae. This study provides data for the assessment and regulation of Carbo-Iron nanoparticles in fish in situ remediation and contributes to the development of reference methods in testing nanomaterials. We additionally provide data useful for the ecotoxicological evaluation of the environmental contaminant PCE.

**WE 205**
Silver nanoparticles: different toxicity mechanisms in Vibrio fischeri and Pseudokirchneriella subcapitata using different kinds of nanoparticles

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In recent years the market for nanotechnology-based consumer products has been rising. Silver nanoparticles (AgNPs) are widely used in several consumer products and they are mainly designed to release silver ions (Ag⁺), the source of antibacterial activity. Unconventional features result from AgNPs small size and large surface area per unit mass, eventually generating significant differences between the physicochemical properties of bulk and nanoparticulate forms of Ag. Ionic silver (Ag) is one of the most toxic forms of metals in the aquatic environment. The release of Ag⁺ from AgNPs will depend on both solution and nanoparticle characteristics, including the composition of nanomaterials and size. The main goal of this study is to assemble and evaluate the toxic effects of citrate-stabilized AgNP solution (100 nm) and non-stabilized AgNP solution (100 nm) and AgNO₃, to bacteria (Vibrio fischeri) and algae (Pseudokirchneriella subcapitata). To determine the biomembranes inhibition rate, V. fischeri was incubated at 15°C and exposed to both kinds of AgNPs for 5 minutes, according to Microtox® test procedures. Algae were exposed to nAg or AgNO₃ in MBL medium and and exposure duration was 72 h. The growth rate was determined according to OECD Guideline 201. At the same time, DLS (Dynamic Light Scattering) was used to measure particle size distributions and the zeta potential of AgNPs. The DLS results demonstrate that non-stabilized AgNP solution (100 mg/L) had a stable zeta potential (-35 mV) for 72 h. The V.fischeri and Pseudokirchneriella subcapitata results show that there is an influence of the surface composition in the toxicity of AgNPs to these aquatic organisms. A comparison of the nanoparticles was made to Ag⁺ toxicity. The implications of manipulation of AgNP surface composition on the toxicity assessment and its ecological relevance will be discussed.

**WE 206**
A comparative study on the influence of physicochemical properties to particle uptake and elimination in Daphnia magna

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Monitoring the distribution and subsequent effects of nanoparticle (NP) contaminants in aquatic ecosystems will be pivotal to developing regulations that minimize the environmental footprint. To answer the question of distribution, our research was designed to characterize the influence of particle size, shape and surface chemistry on uptake and elimination of gold nanoparticles (AuNPs) in a primary consumer. Uptake and elimination rate constants were derived empirically for citrate coated spheres (20 and 30 nm), and polycrylic acid and poly/allylamine hydrochloride coated rods (18 x 58 nm) using Daphnia magna (a pelagic filter-feeding crustacean) as a model organism. Differences in uptake rate constants was most pronounced when the surface coating was varied, with positive coatings exhibiting the highest uptake rate constants of all the treatments. Size and shape, however, did not appear to impact uptake. Elimination by D. magna followed the two-compartment model for each particle configuration predicting that the uptake process will control the concentration in the organisms after t days of exposure. Significant differences between elimination rate constants were observed for particles varying in size but not for particles with different shapes or surface chemistry. Further study of the gut-lumen interface revealed that particles are in contact with the microvilli and in some cases adsorbed to the surface, which could represent the slow exchange compartment. Images, however, could not confirm acculation of particles outside of the gut tract, though this does not preclude the possibility that particles are localized in other compartments.

**WE 207**
Effects of TiO₂ nanoparticles on a freshwater bacterial community - influence of DOC and UV radiation

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The rapidly growing industry with the extensive applications reaching from the technical-, medical and research sector over to a wide range of consumer products. Especially the widespread use in consumer products will lead to their release into the environment, particularly into the aquatic environment, as a sink for many anthropogenic contaminants. The nanoparticles special chemical and physical properties are associated with potential toxicological effects and there is still considerable uncertainty concerning their hazard for humans and the environment. In this study, we investigated the influence of titanium dioxide nanoparticles (TiO₂NP) on natural bacterial communities of three Swedish lakes were assessed, taking environmental parameters, such as dissolved organic carbon (DOC) content and UV intensity into account. The hydrodynamic parameter of the TiO₂NP was approximately 75 nm, with a zeta potential of -60 mV. The nanoparticles were found to be stable in MilliQ water and high DOC lake water; however, during the experiments processes were taking place in lake waters with lower DOC concentrations. Significant reductions in the bacterial abundance were found at 100 µg L⁻¹ in higher DOC lakes, and 1000 µg L⁻¹ in the low DOC lake, reflecting the influence of the particles agglomeration state on their toxic potential. However, differences in UV intensity did not influence the observed effects. The absence of induction of reactive oxygen species formation further corroborated the finding that the toxicity is not driven by phototoxic potential of TiO₂NP. Influences of TiO₂NP on the bacterial community composition are currently under investigation. The presence of TiO₂NP was found to have a strong impact on natural lake water bacterial communities, with the toxic potential of the nanoparticles being significantly enhanced in the presence of increasing DOC.

**WE 208**
Effects of silver nanoparticles on freshwater microbial communities

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In this study we investigated the effects of four different silver nanoparticles (AgNPs) widely used in industrial processes on freshwater bacterial communities. The AgNPs display special chemical properties because of their size, shape, composition and electronic structure. These properties lend NPs their functionality, but also present a potential risk for humans due to their small size and surface area per unit mass.

The experiments were performed on laboratory cultures of bacterial communities from freshwater lakes in southern Sweden, which were spiked with a mixture of four different AgNPs. The AgNPs are produced by different companies and have different surface coatings and sizes. The experiments were performed in the presence and absence of light and at different concentrations of the AgNPs.

The results showed that the AgNPs had a significant impact on the bacterial communities. The effects varied depending on the type of AgNP and the concentration used. At low concentrations, the AgNPs had a positive effect on the bacterial community, promoting the growth of certain species. At higher concentrations, the AgNPs had a negative effect, leading to a decrease in bacterial diversity and abundance. The results also showed that the AgNPs were more toxic to bacterial communities in the presence of light, which is consistent with previous studies showing that light can enhance the toxicity of AgNPs.

The study highlights the importance of considering the effects of AgNPs on freshwater bacterial communities, especially in the context of their widespread use in industrial processes.
In a case study with AgNPs, the toxicity to natural freshwater microbial communities was determined. The microbial communities were exposed to AgNPs of different sizes (10, 20, 40 and 50 nm) as well as different coatings (non-coated, citrate coated) in concentrations from 0.1 - 5000 μmol/L except for one silver nanoparticle type (50 nm, uncoated) which was tested in a range from 100 - 10 000 μmol/L. The selected silver nanoparticles were all purchased from commercial producers and distributors (American British Biocell, NanoTrade, Tedpella). To distinguish between particle related effects and effects caused by free silver ions silver nitrate was tested as a reference in corresponding total silver concentrations. The effects on the algal parts of the community was studied through pigment profile analysis with HPLC. The bacterial community composition was analysed by metabolic profiling on so called ecologeprofils (TRADEMARK) containing different carbon sources with a redox dye responding to respiration with the metabolic activity of the bacteria. The metabolic activity is used to measure the colour change as a measure of microbial activity which was measured over time in several intervals for a total time period of 96 h. Inhibition of growth detected as decrease in total biomass was seen in both bacterial and algal communities. All experiments were backed up by analytical measurements, measuring the total silver content, dissolved Ag+ (ultrafiltration) and particle size distribution (Nanosight).

**WE 209**

**Different sensitivity cyanobacteria and protist by silver nanoparticle**


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Of the 1011 Nanotechnology-based consumer products available on the market in August 2009, the products containing silver nanoparticle (SNP) are the largest (25%) and with the highest growing group. This growth of SNP used products is due to its extraordinary usefulness as a broad-spectrum antimicrobial agent. Despite the growing market of silver nanoparticle, little is known about the aquatic environmental effects of widespread use of SNP contained consumer products. Recent reports have shown that SNP is detected in natural environment. Many reports have revealed highly toxic to aquatic organisms, and the United States Environmental Protection Agency has set water quality regulations for water bodies, such as fish and fresh water. The toxicity and exposure data for SNP however are little for prediction environmental risk. Cyanobacteria, algae, protist, and bacteria consist of microbial food web. When the microbial food web is destroyed, aquatic environmental ecosystem should be damaged leading to become seriously environmental problem. Previous studies showed Gram-negative bacteria are more sensitive than Gram-positive bacteria. Algae exposed silver nanoparticles has been reported many articles showed inhibitory was difference every genus. Cyanobacteria and protist however is little report. Selective inhibitory effect is important for thinking environmental risk.

In this study, we try to reveal inhibitory and selective potential of silver nanoparticle on the growth of a cyanobacteria, Microcystis aeruginosa and grazing the cyanobacteria protist, Monas guttula under laboratory condition. And grazing cyanobacteria activity of Monas guttula was analyzed under several concentration of SNP. At 1.0 mg/L of SNP, Monas guttula was perfectly inhibited the growth. At the concentration 10 μg/L, the inhibitory effect on Monas guttula growth showed small, but grazing cyanobacteria activity did not show inhibitory effect. SNP at 10 μg/L and 1.0 mg/L inhibited around 40% and almost 100%, respectively growth of a cyanobacteria, Microcystis aeruginosa NIES 102. This result is more sensitive than Microcystis aeruginosa UTEX 2388 which was inhibited over 80% growth at 1.0 mg/L and 40% growth at 10 μg/L. Therefore, it was considered that selectively inhibitory effect depend on strain level as well as genus level. And growth inhibition and grazing activity inhibition separately are thought is better for predict environmental risk.

**WE 210**

**Influence of metal oxide nanoparticles on the toxicity of selected pharmaceuticals evaluated in the Spiraxos assay**

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**Spirostomum ambulans** is a very convenient protozoan for ecotoxicological studies due to its large size, ease of culturing and high sensitivity to a broad spectrum of toxicants, including metal ions and pharmaceuticals. Moreover, it can be maintained for several days in inorganic medium at pH from 5.5 to 8.0 and at low and high levels of dissolved oxygen.

The goal of the study was the evaluation the toxicity of selected pharmaceuticals in the presence of bulk and nano sized metal oxides TiO₂ and CoO. The concentration of tested pharmaceuticals monitored with HPLC-DAD. Morphological deformations and subcellular changes were observed under EPI-fluorescence microscope. TEM images of exposed nanoparticles have been developed for cytotoxicity to ecotoxicology. The ecotoxicological work is also complemented with cellular studies, thus drawing linkages between in vitro studies and simple organism studies, as well as between approaches developed for cytotoxicity to ecotoxicology.

**WE 211**

**Ecotoxicity of engineered nanoparticles on a test battery of aquatic organism**

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The ecotoxicity of different diameters of engineered silica nanoparticles and polystyrene nanoparticles on a test battery of aquatic organisms representing four trophic levels is investigated in order to better understand how these nanoparticles can adversely affect the environment. Given the increase in the production of engineered nanoparticles, and thus their potential exposure to the environment, the ecotoxicity of nanoparticles has been reported many articles showed inhibitory on the growth of the cyanobacteria, Microcystis aeruginosa.

The goal of the study was the evaluation the toxicity of selected pharmaceuticals in the presence of bulk and nano sized metal oxides TiO₂ and CoO. The concentration of tested pharmaceuticals monitored with HPLC-DAD. Morphological deformations and subcellular changes were observed under EPI-fluorescence microscope. TEM images of exposed nanoparticles have been developed for cytotoxicity to ecotoxicology. The ecotoxicological work is also complemented with cellular studies, thus drawing linkages between in vitro studies and simple organism studies, as well as between approaches developed for cytotoxicity to ecotoxicology.

**WE 212**

**Toxicity of the nanocomposite Carbo-Iron to Danio rerio and Hyallela azteca**

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In this study, we try to reveal inhibitory and selective potential of silver nanoparticle on the growth of a cyanobacteria, Microcystis aeruginosa and grazing the cyanobacteria protist, Monas guttula under laboratory condition. And grazing cyanobacteria activity of Monas guttula was analyzed under several concentration of SNP. At 1.0 mg/L of SNP, Monas guttula was perfectly inhibited the growth. At the concentration 10 μg/L, the inhibitory effect on Monas guttula growth showed small, but grazing cyanobacteria activity did not show inhibitory effect. SNP at 10 μg/L and 1.0 mg/L inhibited around 40% and almost 100%, respectively growth of a cyanobacteria, Microcystis aeruginosa NIES 102. This result is more sensitive than Microcystis aeruginosa UTEX 2388 which was inhibited over 80% growth at 1.0 mg/L and 40% growth at 10 μg/L. Therefore, it was considered that selectively inhibitory effect depend on strain level as well as genus level. And growth inhibition and grazing activity inhibition separately are thought is better for predict environmental risk.

**WE 213**

**Toxicity and accumulation potential of fullerenes (nC60) in crustacean Daphnia magna in four freshwaters**

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The goal of the study was the evaluation the toxicity of selected pharmaceuticals in the presence of bulk and nano sized metal oxides TiO₂ and CoO. The concentration of tested pharmaceuticals monitored with HPLC-DAD. Morphological deformations and subcellular changes were observed under EPI-fluorescence microscope. TEM images of exposed nanoparticles have been developed for cytotoxicity to ecotoxicology. The ecotoxicological work is also complemented with cellular studies, thus drawing linkages between in vitro studies and simple organism studies, as well as between approaches developed for cytotoxicity to ecotoxicology.
The rapid development of nanotechnology and the increasing use of nanomaterials (NM) have been raising concerns about their fate and potential effects in the environment, since they may have applications as fuels catalyst for which release to aquatic compartment is therefore expected. Besides, ceria NPs are also one of the selected NPs for priority testing by the OECD. Chronic ecotoxicity was investigated on C. dubia following the standardized methods (ISO 20665). The rapid development of nanotechnology and the increasing use of nanomaterials (NM) have been raising concerns about their fate and potential effects in the environment, since they may have applications as fuels catalyst for which release to aquatic compartment is therefore expected. Besides, ceria NPs are also one of the selected NPs for priority testing by the OECD. Chronic ecotoxicity was investigated on C. dubia following the standardized methods (ISO 20665).
In brief, preliminary single dose-response curves of *D. magna* and *D. rerio* to ZnO-NP and UV radiation were taken into consideration to design the combined exposures. The dispersions were prepared by means of ultrasonication with a microtip. Afterwards, the particles were characterized by means of dynamic light scattering and TEM imaging. A range of varying NOM concentrations and AgNO₃ were employed to investigate the effects of varying amounts on nanoparticle size distribution and potential toxicity. Despite this, the risks associated with engineered nanoparticles (ENPs), in the environment are becoming a significant concern. As silver is often found in environmental stressors like high UV radiation levels are factors that have been described as harmful to aquatic organisms. Because ZnO-NP has already been shown to be toxic to a few model species and considering that one of its major applications is as UV filter, the aim of this study was to investigate the joint effects of UV radiation and ZnO-NP to *Daphnia magna* and *Danio rerio* embryos. In brief, two dose-response curves of *D. magna* and *D. rerio* to ZnO-NP and UV radiation were taken into consideration to design the combined exposures. The organisms were exposed simultaneously to UV radiation and ZnO-NP for short time periods followed by a continuous exposure to ZnO-NP. The endpoints analyzed for *D. magna* were survival (OECD 202), feeding inhibition and reproduction (OECD 211) whereas for *D. rerio* the embryonic development was daily checked for any abnormalities and the combined effects were investigated on the Motox model, and assuming that UV radiation and ZnO-NP have dissimilar modes of action on both species, the Independent Action model was used and possible deviations to synergism/antagonism, dose-ratio and dose-level evaluated.

**WE 221**

**Influence of hardness, humic acids and temperature on the lethal toxicity of gold nanoparticles to *Daphnia magna***


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The potential of nanoparticles (NP) to cause adverse effects on biota has been widely reported. However, most works were carried out under standard laboratory conditions and on model marine and freshwater species. Although the results of the experimental tests are relevant for basic research, it is expected that, in the environment, NP will occur under different and varying conditions of several abiotic factors, which will influence their fate and toxicity. Therefore, it is important to understand how such factors will change their bioavailability and toxicity. Accordingly, the present work aimed to evaluate the influence of three abiotic factors (temperature, T; hardness – HAR; and humic acids – HA) on the lethal toxicity of gold nanoparticles (Au-NP) to the cladoceran of *Daphnia magna*. To attain this goal, neonates of *D. magna* were exposed, for 48h, to five concentrations of Au-NP (3.8 to 7.9 µg/L) plus a control (ASTM medium), under three different levels of HAR (180, 90 and 45 mL/1 CaCO₃; hard-standard media, moderately hard, and soft, respectively), HA (1, 3 and 5 mL/L; low; medium and high, respectively) and T (15, 20 and 25OC; low, medium-standard condition and high, respectively). At the end of exposure, immobilised organisms were counted at each test vessel. For each treated test, NP suspensions were characterized, using light scattering: zeta potential (electroophoretic mobility based on Doppler effect), hydrodynamic diameter (Dynamic Light Scattering). Temperature did not change in the measured parameters of NP-Au suspensions. As a decrease in HAR caused a decrease in hydrodynamic diameter and an increase in zeta potential concerning the surface charge of Au-NP, an increase in Au-NP concentration caused an increase in T caused an increase in toxicity of Au-NP, as reflected by the computed L50,48h: 3.2, 4.0, and 9.1 µg/mL of NP-Au, respectively for 25, 20, and 15°C. An increase in HAR did not cause a significant change in lethal toxicity of Au-NP. Finally, increasing concentrations of HA provoked a decrease in lethal toxicity: 6.1, 6.0, 9.1 µg/mL NP-Au, respectively for 1, 3, and 5 µL/L of HA. The results obtained showed the high influence that some abiotic factors may have on the toxicity of NP-Au, highlighting the need to fully understand these processes in order to avoid the underestimation of risk that NP may pose to the environment.

**WE 222**

**Ecotoxic evaluation of zero-valent iron nanomaterials in the aquatic environment**

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New environmental technologies emerge with the development of nanotechnology. The use of zero-valent iron nanomaterials (NZVI) is promising to remediate ground water contaminated with a variety of common environmental pollutants, including chlorinated organic solvents, organochlorine pesticides, and polychlorinated biphenyls (PCBs). The reduction of organic contaminants is a progress successfully applied in the passive barrier remediation technique with micro scale zero-valent iron. A benefit of nanomaterials is the possibility to directly inject them into the contaminated source by in situ remediation technologies. The project NAPASAN funded by the German Federal Ministry of Education and Research aims to develop zero-valent iron nanomaterials that are cost efficient and have good transport qualities in the groundwater aquifer. A key aspect of new technologies in their safe and beneficial application in the field. In this project, the technology is evaluated to ensure the suitability of NZVI for safe remediation. The untreated release as well as potential entrainment of this iron nanomaterial into the environment or reservoirs might lead to the partitioning of iron transformation products to surface waters. Reference nano-Au and bulk iron materials were subjected to standardized acute aquatic and mechanistic ecotoxicity tests: the *Daphnia magna* acute immobilization test, algae growth inhibition test, Ames fluctuation test with cytotoxicity evaluation, and the fish embryo toxicity test. Dose–response curves of Au-NP were generated to determine the acute EC₅₀ values. The EC₅₀ values of Au-NP were 0.84 µg/L, 5.24 µg/L, and 8.67 µg/L for 1, 3, and 5 mg/L of HA, respectively for 1, 3, and 5 µL/L of HA. The results obtained showed the high influence that some abiotic factors may have on the toxicity of NP-Au, highlighting the need to fully understand these processes in order to avoid the underestimation of risk that NP may pose to the environment.

**WE 223**

**Long-term effects of nanosilver - a multi-generation experiment with different Daphnia species**

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The formation and toxicity of silver nanoparticles generated in the presence of natural organic material (NOM) to the cladoceran *Daphnia magna*, *D. pulex* and *D. galeata*. Toxicity tests were conducted with nanosilver (NM-300 silver; particle size 15 nm) from RAS GmbH provided by the OECD sponsorship programme. The results show that silver nanoparticles (10 nm and 25 nm) induced higher toxicity than the 25 nm AgNO₃ solutions. The EC₅₀ values range from 5.7 to 15.7 µg/L depending on the method used to disperse the 25 nm ceria suspensions, whereas EC₅₀ from 1.2 to 1.6 mg/L were recorded after exposure to the 10 nm ceria suspensions. This result highlights that the primary particle size is an important parameter focusing on nanosilver toxicity and the influence of microinvertebrates will also be discussed. In addition to these results, histological and ultrastructural investigations of target cells in *D. magna* (i.e. midgut epithelium) were performed by TEM. The results obtained have clearly shown that the ceria NP were not ingested by the digestive tract lumen of *D. magna*. Disappearance of the apical microvilli of intestinal cells and reduction of rough endoplasmic reticulum network were observed. These ultrastructural damages are sufficient to disturb cellular metabolisms such as nutrient absorption, protein synthesis or phospholipid assembly.

**WE 224**

**The formation and silver toxicity of silver nanoparticles generated in the presence of natural organic material**

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Silver nanoparticles are one of the most widely used engineered nanomaterials [NMs] and their wide spread in consumer products due to its antibacterial properties have made them a potentially important risk assessment and risk management issue. Silver nanoparticles have already been identified as a possible health and environmental adverse effects. Despite this, the risks associated with engineered nanomaterials (ENPs), in the environment are becoming a significant concern. As silver is often found in the wastewater effluents and medical facilities there is a risk that aquatic habitats receiving such effluents and containing natural organic materials (NOM) could provide suitable conditions for the generation of silver nanoparticles in situ. In this study the generation of silver nanoparticles by reduction in the presence of environmentally relevant levels of NOM is described under varying relevant conditions (temperature, pH etc.). This study aimed to develop the methodology to generate NOM functionalized silver nanoparticles and investigate the toxicity of particles generated under differing conditions. Silver nanoparticles were synthesised by the reduction of silver nitrate in the presence of two different NOMs, Swannacer River and Nordic Lake NOM. In this study the production of silver nanoparticles employing sewage effluent containing NOM was also conducted. A range of varying NOM concentrations and AgNO₃ were employed to investigate the effects of varying amounts on nanoparticle size distribution and potential toxicity. A thorough characterisation of all silver nanoparticles was conducted employing Dynamic Light scattering (DLS) for size measurements and zeta potential analysis.
potential, X-ray absorption spectroscopy (XAS) and Transmission Electron Microscopy (TEM). The generated nanoparticle toxicity was then assessed employing standardized ecotoxicity acute and chronic tests for Daphnia magna.

WE 225

The acute test with Daphnia magna underestimates environmental risks of iron oxide nanoparticles
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Iron and iron oxide nanoparticles (INP) are increasingly used for the in situ remediation of contaminated groundwater and soil. Made of zero-valent iron (ZVI), these INP have a high redox potential and are able to reduce many organic compounds to less dangerous metabolites very effectively. Furthermore, they can bind heavy metal ions. In contrast to conventional remediation techniques, application of INP is extremely cost-effective. Therefore, these particles will be applied on many contaminated sites in the future. So far, an environmental risk assessment has not been made. The possible risks could be direct effects (e.g. oxidative stress via iron) as well as indirect effects (INP as co-contaminant for organisms).

ZVI is quickly processed to iron oxides. Therefore, IONP were used in this study. Being less reactive, IONP are also easier to handle. The IONP (Fe$_3$O$_4$) were functionalized with Polyvinylpyrrolidone (PVP) to stabilize them and to prevent them from agglomerating in the medium (Elendt M7). To investigate direct and indirect effects, single and combinatory acute tests with IONP and different potential target contaminants (Cadmium, Copper, Glyphosate, Resorcin) in concentrations up to 100 mg/L were made. Furthermore, the influence of residues from the synthesis on the toxicity of IONP was tested. A quantitative accumulation was measured with a photometric assay, while the qualitative uptake was investigated via histology and Pearls' staining. The study clearly shows potential risks of IONP when released to the environment. Therefore, the use of ZVI and other iron nanoparticles has to be considered thoroughly. Risk regulators for remediation purposes should take into account the limitations of acute tests for assessing environmental hazards.

WE 226

Stability of citrate, PEG-SH and PVP Silver nanoparticles in toxicity media
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Many toxicology studies of silver nanoparticles (AgNPs) to a range of organisms have been performed since the discharge potential of AgNPs to the environment is to be expected. However there has not been any consistent result. Mechanism underlying their toxic effect has also not been fully understood because of information lacking on properties of AgNPs both in pristine suspension and especially in toxicity media used for exposure study. In fact, the media used for exposure study may affect the nature and dose of toxicant. In this study, monodisperse citrate, PEG and PVP coated AgNPs with a core size of approximately 10 nm were synthesised and characterised both in solution as well as in standard OECD media used for Daphnia sp. acute and chronic tests and fish embryo toxicity assay in anaerobic plasma membrane, suspension, cell size, aggregation and shape of AgNPs in those media were monitored and used as indication of stability of AgNPs in toxicity media.

WE 227

Is there a risk of iron (oxide) nanoparticles used for remediation? A case study on combinatorial effects in Daphnia magna
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Iron and iron oxide nanoparticles (INP) are increasingly used for the in situ remediation of contaminated groundwater and soil. Made of zero-valent iron (ZVI), these INP have a high redox potential and are able to reduce many organic compounds to less dangerous metabolites very effectively. Furthermore, they can bind heavy metal ions. In contrast to conventional remediation techniques, application of INP is extremely cost-effective. Therefore, these particles will be applied on many contaminated sites in the future. So far, an environmental risk assessment has not been made. The possible risks could be direct effects (e.g. oxidative stress via iron) as well as indirect effects (INP as a co-contaminant for organisms).

The IONP, consisting of Fe$_3$O$_4$, were functionalized with Polyvinylpyrrolidone (PVP) to stabilize them and to prevent them from agglomerating in the medium (Elendt M7). To investigate direct and indirect effects, single and combinatory acute tests with IONP and different potential target contaminants (Cadmium, Copper, Glyphosate, Resorcin) in concentrations up to 100 mg/L were made. Furthermore, the influence of residues from the synthesis on the toxicity of IONP was investigated. Normally, the IONP were separated from the synthesis solvent (Diethylenglycol DEG). The acute test was performed according to the OECD guideline 202 with some adaptations for testing nanomaterials. The test was prolonged to 96 h in 24 multi-well plates and performed in 24-multilwell plates with 10 replicates and 1 neonate per replicate. Nominal concentrations ranging from 1 to 100 mg/L were used. The acute test showed a slight increase in toxicity after 96 h in the highest test concentration of 100 mg/L, which was not significant compared to the control. The reproduction test showed various effects of the IONP. In DWNTs, mortality over 50 mg/L occurred after 5 to 8 days, showing that acute tests might not be appropriate for testing nanomaterials. In lower concentrations reproduction was significantly inhibited by the IONP. This might result out of direct toxicity (e.g. oxidative stress) or indirect effects. E.g. an increase in agglomeration of algae for feeding could be observed, which resulted in blocking the filtering apparatuses of the daphnids. This might influence the feeding and therefore the reproduction test. Therefore, this study confirmed on iron oxide nanoparticles (IONP).

WE 228

The influence of humic acid on the toxicity of double walled carbon nanotubes to Daphnia magna and fish
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Double walled carbon nanotubes (DWNTs) are regarded as an excellent compromise between the single walled carbon nanotubes and multiwalled carbon nanotubes to their potential applications in electronic devices and compounds for water treatment. The anticipated increasing applications of DWNTs will lead to their ultimate release into the environment through wastewater discharges, accidental spills, and during use phase as water treatment devices. However, presently data on the toxicological effects of DWNTs in the aquatic ecosystems is largely lacking, and in this study, we seek to elucidate their effects to aquatic organisms at different trophic levels. Natural organic matter (NOM) fractions like humic acid are among the major influencing abiotic factors for the toxicity of various toxicants in the aquatic ecosystems. Therefore, in this paper, we investigated the acute toxicity of DWNTs in the aquatic ecosystems.

Here, acute toxicity tests for evaluating the effects of DWNTs in aquatic system were; the algal growth inhibition test, water flea (daphnia) acute toxicity test, and the fish acute toxicity test. The effective concentration (EC50) of DWNTs on algae, daphnia and fish were found to be 11.2 mg/L, 4.49 mg/L, and 374 mg/L respectively, in the acute test with Daphnia magna underestimates environmental risks of iron oxide nanoparticles
J.B. Baumann, D. Arndt, J. Filser
Center for Environment and Sustainable Technology, Bremen, Germany

Iron and iron oxide nanoparticles (INP) are increasingly used for the in situ remediation of contaminated groundwater and soil. Made of zero-valent iron (ZVI), these INP have a high redox potential and are able to reduce many organic compounds to less dangerous metabolites very effectively. Furthermore, they can bind heavy metal ions. In contrast to conventional remediation techniques, application of INP is extremely cost-effective. Therefore, these particles will be applied on many contaminated sites in the future. So far, an environmental risk assessment has not been made. The possible risks could be direct effects (e.g. oxidative stress via iron) as well as indirect effects (INP as a co-contaminant for organisms).

The IONP, consisting of Fe$_3$O$_4$, were functionalized with Polyvinylpyrrolidone (PVP) to stabilize them and to prevent them from agglomerating in the medium (Elendt M7). To investigate direct and indirect effects, single and combinatory acute tests with IONP and different potential target contaminants (Cadmium, Copper, Glyphosate, Resorcin) in concentrations up to 100 mg/L were made. Furthermore, the influence of residues from the synthesis on the toxicity of IONP was investigated. Normally, the IONP were separated from the synthesis solvent (Diethylenglycol DEG). The acute test was performed according to the OECD guideline 202 with some adaptations for testing nanomaterials. The test was prolonged to 96 h in 24 multi-well plates and performed in 24-multilwell plates with 10 replicates and 1 neonate per replicate. Nominal concentrations ranging from 1 to 100 mg/L were used. The acute test showed a slight increase in toxicity after 96 h in the highest test concentration of 100 mg/L, which was not significant compared to the control. The reproduction test showed various effects of the IONP. In DWNTs, mortality over 50 mg/L occurred after 5 to 8 days, showing that acute tests might not be appropriate for testing nanomaterials. In lower concentrations reproduction was significantly inhibited by the IONP. This might result out of direct toxicity (e.g. oxidative stress) or indirect effects. E.g. an increase in agglomeration of algae for feeding could be observed, which resulted in blocking the filtering apparatuses of the daphnids. This might influence the feeding and therefore the reproduction test. Therefore, this study confirmed on iron oxide nanoparticles (IONP).

WE 229

Cumulative damage of tungsten carbide nanoparticles with cobalt doping in Daphnia magna
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The effects of tungsten carbide nanoparticles without (WC) and with cobalt doping (WC-Co) in Daphnia magna mobility were compared after 24, 48, 72 and 96h of exposure. After 24h, almost no individuals were immobilized by WC. In contrast, 50% of the individuals were immobilized by 0.89 mM WC-Co. After 96h, the median effect concentration (EC50) was 7.5 mg/mWC and 0.11 mM WC-Co. To investigate the influence of cobalt on the WC-Co toxicity, the effects of the cobalt content in WC-Co and in cobalt chloride (CoCl$_2$-6H$_2$O) were compared. A similar effect was observed after 24h, but the EC$_{50}$ after 96h was two times higher for CoCl$_2$-6H$_2$O than for WC-Co. The decrease of EC$_{50}$ with increasing exposure time was described by a concentration-time-effect model, which combines the rule of Halberg with the Haber and the Hill model. The slope of the curve that describes this decrease was similar for WC and for WC-Co, and was two times higher for the cobalt in CoCl$_2$-6H$_2$O than in WC-Co. The same model was used to extrapolate the EC$_{50}$ to 96 h and performed in 24-multiwell plates with 10 replicates and 1 neonate per replicate. Nominal concentrations ranging from 1 to 100 mg/L were used. The acute test showed a slight increase in toxicity after 96 h in the highest test concentration of 100 mg/L, which was not significant compared to the control. The reproduction test showed various effects of the IONP. In DWNTs, mortality over 50 mg/L occurred after 5 to 8 days, showing that acute tests might not be appropriate for testing nanomaterials. In lower concentrations reproduction was significantly inhibited by the IONP. This might result out of direct toxicity (e.g. oxidative stress) or indirect effects. E.g. an increase in agglomeration of algae for feeding could be observed, which resulted in blocking the filtering apparatuses of the daphnids. This might influence the feeding and therefore the reproduction test. Therefore, this study confirmed on iron oxide nanoparticles (IONP).
The overall outcome from this study highlights the importance of evaluating nanoparticle toxicity effects, considering different sizes and counterparts on relevant endpoints besides survival, such as feeding inhibition and reproduction responses.

**WE 232**
Do titanium dioxide nanoparticles (TiO2) induce adverse effects in the released offspring of Daphnia magna?
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Titanium dioxide nanoparticles (TiO2) are due to their chemical (e.g. photocatalytic) properties widely and increasingly applied. Hence, TiO2 may finally end up in the surface water bodies where they may pose a risk for aquatic ecosystems. Most of so far conducted toxicity studies with TiO2 investigated the acute and chronic toxicity of TiO2 alone. Those studies were not relevant for understanding the outcome of TiO2, since the properties of the TiO2 nanoparticles used in these studies are not representative of the properties of the TiO2 nanoparticles found in the environment.

Zinc oxide nanoparticles (ZnO-NP) are metal oxide nanoparticles known to have a broad spectrum of applications in personal products, coatings and paints. The last fate of these compounds is likely to be the aquatic environment. The aim of this study was to evaluate the effects of Daphnia magna of different sized ZnO-NP and also compare them with the effects of bulk and finer sized TiO2 nanoparticles (ZnO non-nano scale and zinc chloride (ZnCl2), respectively). The toxic effects of these substances were studied on survival, feeding activity and offspring production.

In this work, differences on particle size showed to influence the toxicity of ZnO to Daphnia magna. In addition it was also observed that zinc chloride presented higher toxicity when compared to the others nanoparticles. The overall outcome from this study highlights the importance of evaluating nanoparticle toxicity effects, considering different sizes and counterparts on relevant endpoints besides survival, such as feeding inhibition and reproduction responses.

**EP04P - Greener nanotechnology, an integrative approach to an emerging technology**

**WE 236**
Poly-epson-caprolactone nanoparticles loaded trazine herbicides: safer formulatioins for herbicide application

Uncapped nTiO2 nanoparticles (nTiO2) can be potentially harmful in the environment, and replacing them with other nanomaterials is therefore preferable. This paper presents a novel approach to this, using the polyepson-caprolactone (PCL) nanoparticles loaded trazine herbicides (imazapyr and azimethrin). The nanoparticles were characterized by the measurements of encapsulation efficiency (EE), size, mean diameter, polydispersity index and their zeta potential of the nanoparticles. Genotoxicity of the formulations were analyzed using Comet assay, micronucleus test, and alkaline etap test conditions, as well as molecular association. The association efficiencies of the herbicides in the self-assembled nanoparticles were higher than 86%.

**WE 237**
Effect of light condition and particle size on toxicity of nano-TiO2 in Daphnia magna
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Triazine herbicides are the most important class of agricultural chemical ever developed. Because of their extensive use in agriculture, they are often found as a pollutant in rural aquatic environments. In order to reduce their toxicity in the environment, the use of polymeric nanoparticles as carrier systems can be a good alternative to improve their effectiveness and minimize their environmental impacts. The objective of the present study was to characterize and investigate the genotoxicity of poly-epson-caprolactone (PCL) nanoparticles loaded trazine herbicides (imazapyr and azimethrin) in aqueous medium. The nanoparticles were characterized by the measurements of encapsulation efficiency (EE), size, mean diameter, polydispersity index and their zeta potential of the nanoparticles. Genotoxicity of the formulations were analyzed using comet assay, micronucleus test, and alkaline etap test conditions, as well as molecular association. The association efficiencies of the herbicides in the self-assembled nanoparticles were higher than 86%.

**WE 238**
How to measure toxicity of nanoparticles to generate data useful for QSAR models?

The remaining algae was centrifuged and resuspended in MBL medium for depuration phase, which lasted for another 48hrs with sample taken after 24 and 48 hrs. For the Daphnia bioaccumulation assay, 7 day-old Daphnia magna were exposed to two concentrations of AgNO3 and AgNPs for 96 hrs (24 hrs exposure followed by 72 hrs depuration). Bioaccumulation patterns were similar for both algae and daphnia with increasing internal concentration of silver at higher medium concentrations. Algae reached a plateau of internal concentration of silver after 48 hrs exposure of 1 mg/L of either silver nitrate and nanoparticles had a higher body burden of silver. After 48 hrs of depuration, the concentration of silver in Daphnia magna was insignificant when compared to control animals, indicating that complete elimination of silver occurred within the 48 hrs following exposure. Internalization was also one of the parameters used in this study giving patterns for accumulation inside organisms, which were dependent from the exposure used. These results indicate a first insight of bioaccumulation of nanoparticles in comparison with metal ions in algae and daphnia, and will be useful to assess the metal trophic transfer within an aquatic trophic chain.
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However, the realisation of pulse-chase feeding experiments remains complex. The experiment is time and money consuming. The collection of small amounts of faeces in microcosms where organisms are fed is practically difficult and the interpretation of ICP-MS outputs to estimate the net amount of stable isotopes in the samples is not straightforward.

Moreover, inorganic carbon is the assumption of a mass balance of stable isotopes between the one hand, and the mussel and faeces in the other hand. We present several attempts to assess the assimilation efficiency of several metals to zebra mussel, Dreissena polymorpha, using the pulse chase-feeding method. Metal isotopes were $^{56}$Cr, $^{50}$Ni, $^{113}$Cu, $^{109}$Zn and $^{114}$Cd. Several types of several parameters were tested. In most cases, assimilation efficiencies could be estimated from the experimental data with a great uncertainty. However, the metal mass balance could not be balanced, mainly due to water/particles exchanges that cannot be neglected. We propose to discuss the feasibility and the possible artefacts of this method.

**WE 243**
Bioaccumulation studies in the closed laboratory aquatic microcosm AquaHab®

M.D. Dinne$^*$, K.S. Slentzka, B.J. Jastorff$^1$, I.S. Stan$^2$

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AquaHab® is a small aquatic microcosm on laboratory scale for the prospective risk assessment of chemicals. The 7.5 L water tank contains different groups of organisms (fish, invertebrates, bacteria etc.) and conditions are highly standardized and test results are highly reproducible due to an automated measuring and regulation unit (regulating temperature and [O$_2$] concentration in water) as well as isolation from external environmental conditions.

In addition to the possibility of studying effects on different levels of biological organization (from subcellular endpoints to system endpoints) in species of different trophic levels the system is suitable for long term exposure analysis of chemicals, as past studies withCr(VI)-TBT, Legard 1990 and ionic liquids have demonstrated.

The bioaccumulation and transformation of chemicals in the different groups of organisms can be studied.

Bioravable trophic levels of organisms implement different mechanisms for uptake, transformation and excretion of chemicals by organisms into the exposure assessment, as given in the model. Samples from water and sediments could also be taken during test runs, which enables eco-temporal analysis. A further major advantage is the possibility to study bioaccumulation under competition of different groups of organisms for the chemical.

The integration of sediment as an important environmental compartment is planned as the next step of development.

**WE 244**
Methods for testing the ecotoxicity of ionizing compounds at multiple pH using Daphnia magna

C. Rendal, S. Dalgaard, O.K. Kusk

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Many of the emerging environmental contaminants that have recently come into focus have one thing in common: they are ionizing compounds. An evaluation of a random sample of the over 140000 chemicals pre-registered for REACH revealed that 27% were acids 14% were bases 8% were zwitterions. Risk assessment of these ionizing compounds is complicated due to the different behaviour of the neutral and ionized molecules. Neutral molecules are generally more lipophilic, and will be taken up by organisms to a greater extent than ions. Ions are subject to electrical attraction or repulsion, and the uptake of ionizing compounds is further complicated by processes such as the ion trap and the differential effects of high cellular ionic strength on the activity of neutral and ionized molecules.

The bioavailability and bioaccumulation of molecules is dependent on the pH of the compound, but also on the pH of the test solution. Acids are more neutral (and thus probably more toxic) at pH levels below their $pK_a$, while bases are more neutral (and thus probably more toxic) at pH levels above their $pK_a$. A recent review recommended that bases be tested for toxicity at pH levels above their $pK_a$, acids at pH levels below their $pK_a$, and zwitterions at the isoelectric point. This recommendation calls for modification of existing test methods to require tests to be carried out over a more restricted pH range.

Based on a literature review of the methods previously used to buffer solutions in ecotoxicological tests, we selected eight buffers for further investigation, namely TRIS, MES, HEPES, MOPS, CAPS, CHE, sulfate and citric acid. The buffers were tested for toxic effects using the 48 acute immobilization test with Daphnia magna at various pH levels. In that test it was determined that the pH level of the exposure solution, buffers and working concentrations can be recommended for at least pH levels with a pH drift of no more than ±0.2 pH units, and a buffer toxicity of at least a factor three below EC10.

Different test conditions could be studied by testing with same species with different biomasses, different application forms of the chemicals (e.g. via water or partly via food) at other constant conditions - the influence of ecological variation on bioaccumulation could be studied e.g. by changes in the food web, changes in the composition of species (e.g. predator-prey relationships).

Results of these basic studies with the above mentioned chemicals (e.g. TBT) will be presented on the poster, as well as an outlook to future studies.

**WE 245**
A benchmark approach to determine absorption efficiencies in fish

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We present a novel method for measuring fish gut efficiencies of organic chemicals using a benchmarking approach. Fish were given feed contaminated by test substances having log Kow-values ranging from 4 to 10, including several benchmarking chemicals. The benchmarking chemicals, three PCBs and decabromodiphenoxyethylen, were given a single dose to cover a range of molecular weights and ionization properties.

Acids were fed at pH 1.5 and bases were fed at pH 4. The uptake of ionizing compounds is further complicated by processes such as the ion trap and the differential effects of high cellular ionic strength on the activity of neutral and ionized molecules. The bioavailability and bioaccumulation of molecules is dependent on the $pK_a$ of the compound, but also on the pH of the test solution. Acids are more neutral (and thus probably more toxic) at $pH$ levels below their $pK_a$, while bases are more neutral (and thus probably more toxic) at $pH$ levels above their $pK_a$. A recent review recommended that bases be tested for toxicity at $pH$ levels above their $pK_a$, acids at $pH$ levels below their $pK_a$, and zwitterions at the isoelectric point. This recommendation calls for modification of existing test methods to require tests to be carried out over a more restricted $pH$ range.

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in the Hepa1c1c7 cell line. The aim is to quantify the uptake and distribution of BaP into the cell-, media- and the plastic fraction of the exposure scenario over time. According to a time- and concentration dependent analysis of the CYP1A1 enzymatic activity (concentration range: 0.5 nM to 5 µM BaP; time points: 2, 4, 12 & 24 hours [h]), at least 12h of BaP exposure are required for the enzyme to be fully active. Further, a method was established to determine the cell internal concentration by using 14C-BaP as an internal standard. The same samples were measured by liquid scintillation counting for the analysis of total radioactivity and by high performance liquid chromatography (HPLC) and radio-HPLC to detect the presence of metabolites. The results of the analysis revealed absence of detectable metabolites at 2 and 4h, which agrees with measured CYP1A1 enzymatic activity. After 2h of exposure, 5.0-6.5% of the nominal BaP (75 nmols) was found in the cells, 76-96% remained in the media, and 0.2-4.7% was adsorbed to the plastic (with cell number as constant). BaP in the medium fraction was reduced, while in the plastic fraction stayed constant for both time points, indicating that the BaP adsorbed to the plastic was not readily bioavailable. The cell internal concentration after 2 and 4h was calculated as 0.3-0.7 and 0.8-1.1 pg/cell, respectively. Preliminary results show formation of metabolites at 12 and 24h. Further adjustments are currently being performed to optimize the determination of BaP metabolites.

WE 249

Multi-species comparison of the mechanism of MeO-BDEs to OH-BDEs in fish M. Hecker1, F.Y. Liu1, S.B. Wiseman1, Y. Wan1, J.P. Doering1, M.H.W. Lam1, J.P. Giesy1

1University of Saskatchewan, Saskatoon, Canada
2City University of Hong Kong, Hong Kong, China
3Polybrominated diphenyl ethers (PBDEs) and their methoxylated (MeO-B) and hydroxylated (OH-) analogs are ubiquitously distributed in the environment worldwide. The OH-BDEs are more potent than PBDEs for many endpoints and can be produced from the transformation of MeO-BDEs. The objectives of the current study were to (1) identify the enzyme(s) that catalyze the biotransformation of 6-MeO-BDE-47 to 6-OH-BDE-47 in livers from rainbow trout, and (2) compare transformation of 6-MeO-BDE-47 to 6-OH-BDE-47 among rainbow trout, white sturgeon and goldfish. Cytochrome P450 1A (CYP1A) enzymes did not catalyze the transformation reaction. However, transformation was inhibited by the CYP inhibitors clomethiazole and 1-benzylimidazole but not gestodene. Therefore, the reaction is likely catalyzed by CYP2 enzymes. When transformed, 6-MeO-BDE-47 was oxidized among species, concentrations of 6-OH-BDE-47 were 3.4 and 9.1 times greater in microsomes from trout compared to goldfish and sturgeon, respectively. Concentrations of 6-OH-BDE-47 in microsomes from goldfish were 2.7 times greater than in sturgeon. The initial rate of transformation in microsomes from trout was 2.9 and 6.2 times greater than that of goldfish and sturgeon, respectively, while the initial rate in goldfish was 3.1 times greater than in sturgeon. Overall, differences in CYP mediated transformation of MeO-BDEs to OH-BDEs could influence concentrations of OH-BDEs in different species of fish.

WE 250

Compound lipophilicity as a descriptor to predict metabolic affinity (Km) in mammals A.P. Konieczny1, A. Pirovano2, M.A.J. Huijbregts3, I.A. O’Connor4, A.M.J. Ragas4, A.J. Hendriks5

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Lipophilic chemicals have the potential for high membrane affinity and increased bioaccumulation across lipid membranes. Traditional physicochemical methods use the octanol-water partitioning coefficient (Kow) as the standard for predicting chemical uptake and bioaccumulation. However, octanol does not accurately reflect the phospholipids and proteins found in cellular lipid bilayer membranes. Newly developed methods utilizing phosphatidylcholine is a better surrogate than octanol for cellular membranes because of the natural presence of phosphatidylcholine in cell membranes, improved membrane fluidity, and improved range of complex interactions between the chemicals and membrane molecules. Thus, this new technique was utilized to evaluate the bioavailability of legacy nonionizable chemicals (2,4,6-trinitrotoluene [TNT], hexahydro-1,3,5-trinitro-1,3,5-triazine [RDX], 2,4-dinitrotoluene [2,4-DNT]), as well as newly developed munitions (2,4-dinitroanisole [DNA], 5-nitro-2,4,6-triazole-3-one [NT3O]), solid rocket motor propellants (propellant M5 and M7), and a PAGA-bound ester. These models were compared with 1 mlg/micronutrient measurements in the manufacturer’s instructions. Samples were centrifuged and the supernatant was removed for chemical analyses. The chemical membrane affinity (also referred to as lipid-water partition coefficient [Kow]) was calculated according to the manufacturer’s instructions. Results demonstrated that most Kow values for these compounds are smaller than their Kow values, and these differences validate the use of these models in their metabolite profiles. In vivo bioavailability (through biological fluids (e.g., gastric, intestinal)) to see if there are any changes in bioavailability due to organ-specific environments. These data will assist scientists and risk assessors better determine the potential of chemical bioavailability.

WE 251

Membrane affinity of munitions compounds and environmental breakdown products D.R. Johnson1, A.B. Goins2, C.Y. Ang3, T. Reese4, A.J. Bednar5, F.C. Hill6

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Lipophilic chemicals have the potential for high membrane affinity and increased bioaccumulation across lipid membranes. Traditional physicochemical methods use the octanol-water partitioning coefficient (Kow) as the standard for predicting chemical uptake and bioaccumulation. However, octanol does not accurately reflect the phospholipids and proteins found in cellular lipid bilayer membranes. Newly developed methods utilizing phosphatidylcholine is a better surrogate than octanol for cellular membranes because of the natural presence of phosphatidylcholine in cell membranes, improved membrane fluidity, and improved range of complex interactions between the chemicals and membrane molecules. Thus, this new technique was utilized to evaluate the bioavailability of legacy nonionizable chemicals (2,4,6-trinitrotoluene [TNT], hexahydro-1,3,5-trinitro-1,3,5-triazine [RDX], 2,4-dinitrotoluene [2,4-DNT]), as well as newly developed munitions (2,4-dinitroanisole [DNA], 5-nitro-2,4,6-triazole-3-one [NT3O]), solid rocket motor propellants (propellant M5 and M7), and a PAGA-bound ester. These models were compared with 1 mlg/micronutrient measurements in the manufacturer’s instructions. Samples were centrifuged and the supernatant was removed for chemical analyses. The chemical membrane affinity (also referred to as lipid-water partition coefficient [Kow]) was calculated according to the manufacturer’s instructions. Results demonstrated that most Kow values for these compounds are smaller than their Kow values, and these differences validate the use of these models in their metabolite profiles. In vivo bioavailability (through biological fluids (e.g., gastric, intestinal)) to see if there are any changes in bioavailability due to organ-specific environments. These data will assist scientists and risk assessors better determine the potential of chemical bioavailability.

WE 252

BCF base-line model: further concept and application developments D.G. Georgiev1, S. Dimitrova1, T. Hartfield2, J. Straka1, O. Mekenyan1

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The study describes further developments of the BCF base-line model focused on expanding its training set and applicability domain, re-formulation of mitigating factors and re-evaluation of model parameters. Software implementation of the model allows prediction of BCF based on chemical structure and observed or calculated input parameters. The model was re-evaluated for the most common chemical classes in the fragrance industry such as esters, alcohols and ketones using rainbow trout ( Oncorhynchus mykiss) liver 59 fractions to assess their bioaccumulation potential. In addition to the determination of the enzymatic turnover rates, we investigated the impact of individual cofactors (NADPH, UDPGA, PAPS, GSH) on the metabolic stability of the test substances to provide insights into the potential metabolic routes.

WE 253

Assessment of bioaccumulation potential under REACH combining classification rules derived from physicochemical and structural information
A database consisting of 844 distinct organic chemicals was created. In addition to the CAS number and the canonical SMILES, it contains information on nine descriptors indicating that the bioaccumulation potential should be limited. However, less available than PAHs of creosote oil. This may explain the higher accumulation of creosote oil based PAHs. Part of the bioaccumulation results may be explained by the association rules extracted from frequent sets using structural information.

Two-year-old marbled soles (Pseudopleuronectes yokohamae) (avg. 46 g-wet, n = 89) were held in a flow-through system of filtered seawater (avg. water quality: 17.3 °C; 7.5 mg O2/L). There were control, water (W), bottom-sediment (BS), and suspended-sediment (SS) treatments (T) (control and WT: no sediment; BST: spiked BS; SST: no BS). Only the WT received spiked seawater. Spiked field-collected BS had been laid in the BST at 1-2 cm thick and was not renewed during the experiment. SS was also present in the BST due to the activity of the fish. The SST received contaminated from the BST. An 84-d detoxication period followed a 28-d exposure period (only exposure period for SST). Some water and sediments were sampled regularly and analyzed for PFOs. The fish body surface, gills, and inside of gut were washed to remove particles. Uptake and depuration kinetics of PFOs from water in a marine fish were investigated with and without sediment, and potential impact of the sediment on the kinetics was evaluated.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous lipophilic contaminants that accumulate and persist in sediments and thus can be taken up by sediment dwelling organisms. The sources of PAHs varies from petrogenic to pyrogenic, and the source is an important estimator for bioavailability; the bioaccumulation of petrogenic PAHs has been a subject of research. A number of studies have been carried out to verify that of pyrogenic PAHs. The PAHs are typically stored in the body by the lipid membranes, and black carbon content, of the sediment affect the bioavailability of PAHs. In this study the role of PAH origin and sediment carbon content in bioavailability was assessed by exposing sediment dwelling oligochaetes Littoribius variegatus to four sediments contaminated by either creosote oil or combustion based pyrogenic PAHs. The chemical methods (polyoxymethylene stripes and Tenax-TA tubes) were used for estimating the bioavailability of PAHs. Kinetic bioaccumulation of PAHs and feeding behavior of L. variegatus were determined. Preliminary results show that the sediments contaminated with creosote oil had lower organic and black carbon content and thus the bioavailability was expected to be higher than sediments contaminated with pyrogenic PAHs. The slightly higher Log Koc values of creosote oil based PAHs indicated that the bioavailability of creosote oil based PAHs could be lower than PAHs from other pyrogenic source. The rapidly desorbing fraction, which is considered to be the bioavailable fraction, was very small in all sediments indicating that the bioaccumulation potential should be limited. However, L. variegatus accumulated PAHs and accumulation was higher from creosote oil contaminated sediments than sediments of pyrogenic origin. Due to the very tight absorption of pyrogenic PAHs to black carbon during combustion the pyrogenic PAHs probably are less available than PAHs of creosote oil. This may explain the higher accumulation of creosote oil based PAHs. Part of the bioaccumulation results may be explained by the higher feeding activity of L. variegatus in creosote oil contaminated sediments.

Microcystins (MCs) are cyclic heptapeptide produced by cyanobacteria. There are over 100 different MC congeners reported up to now, having different toxicity. MC-LR is by far the most studied and ubiquitous toxin, with know hepatotoxic effects, inhibition of protein-phosphatases 1 and 2-A, which promotes cancer and other diseases. We investigated the uptake, accumulation and detoxication of Microcystin-LR in shrimps (Palaemonetes argentinus) during 3 days and then translated to new tanks without MCs for additional three days. Crustaceans presented bioaccumulation after 3 days exposure (0.74 ± 0.15 µg MC-LR g wet body-1). The uptake rate constant was estimated at 0.023 [d] (95% confidence interval 0.019-0.026). Uptake rate constant from the dissolved phase was estimated at 22 [L/kg-wet g] (20-25), 30 (24-40), and 46 (39-34) for the WT, BST, respectively. Several factors that may affect the rate constant of uptake, including sediment particles in fish, dissolved oxygen, ingestion and sediment particles, did not explain the magnitude of the increase in the presence of sediment. The cause of this increase needs further investigation.

The bioavailability of different pyrogenic PAHs in field contaminated sediments with phenol adapted population of Lymantria dispar. After quercetin treatment, mortality was higher in unadapted population of Lymantria dispers L. In the caterpillars from phenol adapted population than in unadapted population. An artificial diet with quercetin led to an increase of CAT and GST activities and glutathione-GSH in both populations. The emphasis REACH puts on estimated chemical properties shall reduce experimental testing, thus lowering monetary costs and saving animal lives. The present study aims to classify substances as either B or non-B based on classification rules generated by conformational inference trees using physicochemical properties or generated by association rules extracted from frequent sets using structural information. A database containing 307 chemicals were created. In addition to the CAS number and the canonical SMILES, it contains information on nine descriptors. The various descriptors were estimated with Epi Suite and Open Babel. The entries of the database were cleaned for erroneous CAS, SMILES and BCFF. Additionally a set of 307 chemical structures defining the organic chemical space was used to generate a set of substructures present in each chemical. The descriptors were extracted from chemicals. The descriptors were analyzed for bioaccumulation in terms of added information versus added noise. However, there are different levels of importance among them. For the non-B rules derived from the trees it seems that degradation and metabolization of the compound are driving forces and that for B rules the interaction of the compound with the lipid membranes is more important. The analysis of the structural information in the descriptors revealed that all. The present work contributes to a better understanding of mechanisms and processes leading to low or high BCFs. Classification rules which include either up to seven physicochemical descriptors or one or more of 107 structural fragments discriminate B and non-B chemicals.

The bioavailability of different pyrogenic PAHs in field contaminated sediments was determined in terms of uptake, accumulation and detoxication in shrimps (L. variegatus) during 3 days and then translated to new tanks without MCs for additional three days. Crustaceans presented bioaccumulation after 3 days exposure (0.74 ± 0.15 µg MC-LR g wet body-1). The uptake rate constant was estimated at 0.023 [d] (95% confidence interval 0.019-0.026). Uptake rate constant from the dissolved phase was estimated at 22 [L/kg-wet g] (20-25), 30 (24-40), and 46 (39-34) for the WT, BST, respectively. Several factors that may affect the rate constant of uptake, including sediment particles in fish, dissolved oxygen, ingestion and sediment particles, did not explain the magnitude of the increase in the presence of sediment. The cause of this increase needs further investigation.

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The potential for aquaculture in the Middle East
Lake Greifn sediments were characterized and the temporal resolution of different contaminants were obtained. After the characterization of the lake, a group of chemicals were selected to study their bioaccumulation and effect on Daphnia diapausing eggs. The chemicals were selected based on their physical and chemical properties as well as their occurrence in the lake.

The bioconcentration factors (BCF) of six different chemicals were calculated. The results show that ephippia can take up contaminants from the pore water in the sediment and that the BCF for the neutral compounds are higher with the log Kow values. In addition, the obtained BCF values were compared with values obtained via a QSAR based on log Kow for bioconcentration of organic chemicals in Daphnia, algae, and mussels. The estimated BCF are always higher than the measured ones indicating that Daphnia is not a good model for the bioconcentration in ephippia.

**WE 264**

**Drastic decrease in the organochlorine blood concentrations in nestlings of Goshawk (Accipiter gentilis)**

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Organochlorine pesticides have been the main groups of environmental contaminants as a result of human agricultural activities. These compounds are bioaccumulated and biomagnified in the living organisms in the upper of the trophic chains, and they are able to alter the topography and dynamics of different species. Some studies have related their exposure with impairment of reproductive, endocrine, immune and nervous functions in organisms.

Blood samples from nestlings indicate the degree of short-term exposure determined by the presence of these compounds in the prey that parents bring them to the nest.

The studied area encompassed a montaneous area of Mediterranean forest (Murcia Region, Spain) that is surrounded by agricultural lands (main crops are grapes, almonds, olives, and cereals) and is distant from any urban, industrial, or mining areas. In this area, the goshawk population is sedentary and it has suffered population declines in Murcia Region during the last two decades.

During 2000-2003, all compounds were detected in some samples, being cy clodesine the most frequently detected (100%) in blood. Moreover, these compounds were also the pesticides with the highest blood concentrations. Since 2007, the detection of these pesticides in blood has decreased drastically. DDT and DDE have been detected in concentrations that probably were associated to the agricultural use of dicofol. On the other hand, lindane was not detected in any sample, possibly due to the prohibition in the European Union in 2000 (Decision, 2000/801/EC). In conclusion, we believe that the decrease of the organochlorine blood levels in nestlings of Goshawk have been consequence of the changes in agricultural practices in the growing area and the implementation of legislation on the prohibition of the use of pesticides since 2000.

Acknowledgements: Thanks to the Ministry for Science and Innovation and Seneca Foundation (CARM) for their financial support via MISCA2004 (CGL2004-5595/BOS), MISCA2008 (CGL2008-4318/BOS) and 07383/PIDO projects.

**WE 265**

**Evaluation of three bivalves species as bioindicators of persistent organic pollutants in a Mediterranean coastal lagoon**

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Bivalve molluscs are good bio-indicators of contaminants in surface water by the International Conventions, such as Barcelona Convention and the Oslo and Paris Convention (OSPAR). Bivalves filter great seawater volumes, incorporating dissolved hydrophobic pollutants and accumulating them, being a good bioindicator of these pollutants in seawater. The concentrations measured in the tissue are a function of bioavailable hydrophobic pollutant levels in water. In fact molluscs are commonly used in the short- and intermediate-monitoring programs due to the wide geographical distribution, sessile character and capacity to filter high seawater volumes containing contaminants in their tissue. Mussels are widely distributed, they are not present in many areas such as in the Mar Menor Lagoon, where other bivalve species must be used as bioindicators. Mar Menor is a hypersaline (42-47 psu) coastal lagoon located in the Cartagena Field area at the South East of Spain. This semi-arid area is subject to seasonal rainfall, seasonal tourism, recreational activities and sporadic torrential rain regime. For these reasons, in this study we have characterized the distribution of polycyclic aromatic hydrocarbons, polychlorinated biphenyls and organochlorinated pesticides (OCPs) in three bivalve species. Concretely the bioaccumulation has been determined in cockle, oyster and mussels of a growing area and the potential biomonitoring role of these species has been assessed. Some attempts of this study are to characterize the bioaccumulation of organic pollutants in three bivalve species in the Mar Menor Lagoon, to evaluate the seasonal variation of persistent organic pollutants (POPs) levels in bivalves and to identify the bivalve species most adequate as bioindicators of POPs in Mediterranean coastal areas where mussels were not present. The variability of POPs concentrations in the lagoon was mainly consequence of the sampling area (different hydrodynamic regime and pollution sources), and in less extension of seasonal variations. The bioaccumulation of PAHs is similar for cockle, oyster and mussels, being higher close to ports and wastewater effluents. However, the bioaccumulation of DDDs and PCBs in oyster and P. nobilis is similar in all areas, being higher than detected in cockle. A monitoring programme using both cockle and oyster could be adequate to get representative samples for yearly (cockle) and biyearly (oyster) pollution periods.

**WE 266**

**Environmental monitoring through of linear alkylbenzenes in bivalve molluscs as an bioindicator of exposure to sewage in coastal area, Santos, Brazil**

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The contamination of coastal areas by human activities has been intensified in recent decades. In general, the introduction of contaminants occurs such as domestic and industrial sewage, boats, rivers, atmospheric deposition, agriculture and aquaculture activities intensive in some coastal areas. The discharge of domestic sewage is a major source of pollution in coastal regions. In Brazil it is estimated that only 20% of the population are connected to a wastewater treatment system, often being discarded into the environment without treatment.

The introduction of sewage into aquatic ecosystems can be monitored by different sentinel organisms such as oysters and mussels. One of the organic chemical markers used in most studies are the linear alkylbenzenes (LABs). The main use of LABs is the formation of linear alkylbenzen sulfonated (LAS), used in the formulation of detergents.

The aim of this study was the linear alkylbenzenes in bivalve molluscs (mussels - Mytella falcata and Mytella guyanensis) in a highly urbanized coastal region (Santos, Brazil) as a way to monitor the introduction of sewage in the marine environment. Santos is located in Brazilian southeastern coast, where there are the most important industrial complex of the country and the biggest port of Latin America that have an important contribution of waste disposal can also be found in this region. Eleven samples were extracted with a mixture of hexane / dichloromethane 50% (v/v) and purified on a column of silica and alumina. Identifications and quantifications were made by gas chromatography with mass spectrometry detector (GC/MS).

The LABs concentrations ranged from 110 to 3339 ng g·1 (dry weight), which are comparable to other studies that evaluated the densely urbanized around the world the contribution of sewage.

In relation to individual LABs, samples were more enriched with high input of C13-LAB isomers followed by C12, C11 and C10-LAB. Since the C13-LAB isomers are more hydrophobic than the others, they can be related to the particulate material, and thus be available for the molluscs.

The results of LABs in the bivalve analysed showed they are good bioindicator of sewage input in coastal areas.

**WE 267**

**Development of a field-based technique to assess soil pollution through measurement of contaminant bioavailability to earthworms**

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It is recognised that pollutant uptake by earthworms can be used to interpret results of bioassays. Furthermore, uptake of soil pollutants by laboratory-bred, synchronised earthworms could be used to study the physico-chemical variation of soils, helping to address uncertainties linked to the use of soil screening Values. Therefore, measuring contaminant bioavailability by earthworms could be used in both lower and higher tier assessments within regulatory frameworks for ecological risk assessment.

Moreover, extrapolation of results from laboratory bioassays to field conditions has been widely questioned. This ongoing research aims to develop a mark-release-recapture technique to expose naive earthworms to polluted soil in situ.

The hypothesis is based on two earthworm species, Lumbricus rubellus and Octolasion cyaneum (an epigeic and endogeic species respectively). Field-based experiments using tagged naive earthworms have suggested that:

- Earthworms cannot be retrieved without a form of containment.
- PVC tubes (0.2m diameter, 0.3m length) of 0.1m inserted to a depth of 0.1m, are suitable to prevent movement of earthworms (79% and 84% retrieval for epigeic and endogeic earthworms respectively).

However, such tubes may be disturbed by the public.

- PVC tubes (0.2m diameter, 0.1m, 0.1m, length) flush with the ground, are suitable to prevent subterranean movement of earthworms in unpolluted conditions, but may not prevent over surf movement (79% and 0% retrieval for epigeic and endogeic earthworms respectively). Ongoing experiments are investigating restricting surface movement by covering such tubes with a 0.5mm mesh.

To investigate pollutant avoidance, individuals of the selected earthworm species were introduced into gradients of polluted soil in the laboratory. Results suggested an avoidance of 15% of L. rubellus and 55% of O. cyaneum in unpolluted soil of less polluted soil after one week and, 70% and 90% of L. rubellus and O. cyaneum introduced in polluted soil were recovered in less polluted soil after one week and, 70% and 90% of L. rubellus and O. cyaneum introduced in unpolluted soil remained there. These results emphasise the need for containment of earthworms (in the field) and the potential use of an avoidance response in wider ecotoxicological experiments. Chemical analyses investigating a potential link between soil heterogeneity and uptake of pollutants by earthworms are ongoing. In addition, laboratory-based experiments are being conducted to investigate earthworm movement in response to pollutants using 2D terrestrial.

**WE 268**

**Foliar metal uptake: mechanisms and pathways involved for various plant species**

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1, Y. Foucault

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WE 265
Biodynamic model to predict bioavailable fraction of metals in sediment: the sediment ingesting aquatic oligochaete Lumbriculus variegatus
L. Ardestani1, D.C. Aldridge1, C. Laneiro2, P. Palma2, M. Palma2, A. Cifariello2, M. Ardestani1, D. C. Aldridge1
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2 Polytechnic Institute of Beja - Agricultural Superior School, Beja, Portugal

The aim of this work is to develop a model that can predict the fraction of metals in sediments that is available for uptake by aquatic oligochaetes. This is important because it allows for a better understanding of the bioavailability of metals in sediments and the potential for metal bioaccumulation in aquatic organisms.

WE 270
Interspecific differences in bioaccumulation of heavy metals in three freshwater bivalves
N. Spann1, D.C. Aldridge1, C. Laneiro2, P. Palma2
1 University of Bielefeld, Bielefeld, Germany
2 University of Cambridge, Cambridge, United Kingdom

The study of interspecific differences in bioaccumulation of heavy metals in freshwater bivalves is important for understanding the potential for bioaccumulation and bioavailability of metals in different species. This information can be used to predict the bioavailability of metals in different environments and to inform risk assessment.

WE 271
Biodynamic model to predict bioavailable fraction of metals in sediment: the springing aquatic oligochaete Lumbriculus variegatus
M. Ardestani1, D.C. Aldridge1, C. Laneiro2, P. Palma2, M. Palma2, A. Cifariello2, M. Ardestani1, D. C. Aldridge1
1 Instituto Politécnico de Beja, Escola Superior Agrária, Beja, Portugal
2 Polytechnic Institute of Beja - Agricultural Superior School, Beja, Portugal

The study of bioaccumulation in aquatic oligochaetes is important for understanding the bioavailability of metals in sediments and the potential for metal bioaccumulation in aquatic organisms. This information can be used to inform risk assessment and inform environmental management decisions.

WE 272
The effect of soil pH on copper toxicokinetics in the springtail Folsomia candida
M. Ardestani1, D.C. Aldridge1, C. Laneiro2, P. Palma2, M. Palma2, A. Cifariello2, M. Ardestani1, D. C. Aldridge1
1 Instituto Politécnico de Beja, Escola Superior Agrária, Beja, Portugal
2 Polytechnic Institute of Beja - Agricultural Superior School, Beja, Portugal

The study of the effect of soil pH on copper toxicokinetics in the springtail Folsomia candida is important for understanding the potential for metal bioaccumulation and bioavailability in different environments. This information can be used to inform risk assessment and environmental management decisions.

WE 273
Evaluation of the bioavailability of copper in sediment using an aquatic plant (Myriophyllum aquaticum) and a geochemical approach (DGT)
A. Caillat1, M. Groot1, P. Cifroy2, J.M. Garnier2
1 EDF R&D-Laboratoire National d’Hydraulique et Environnement, Chatsu, France
2 CEREGE, Université Paul Cézanne, UMR 6635 CNRS, Aix en provence, France

The study of the bioavailability of copper in sediment using an aquatic plant (Myriophyllum aquaticum) and a geochemical approach (DGT) is important for understanding the potential for metal bioaccumulation and bioavailability in different environments. This information can be used to inform risk assessment and environmental management decisions.

WE 274
Comparison of the physical and chemical properties of metal-laden sediments from two abandoned mines: the influence of the metal speciation
M. Ardestani1, D.C. Aldridge1, C. Laneiro2, P. Palma2, M. Palma2, A. Cifariello2, M. Ardestani1, D. C. Aldridge1
1 Instituto Politécnico de Beja, Escola Superior Agrária, Beja, Portugal
2 Polytechnic Institute of Beja - Agricultural Superior School, Beja, Portugal

The study of the comparison of the physical and chemical properties of metal-laden sediments from two abandoned mines is important for understanding the potential for metal bioaccumulation and bioavailability in different environments. This information can be used to inform risk assessment and environmental management decisions.
For this purpose we prepared artificial sediments with different properties (variation of the OECD 207-218 normalised sediment, modified by adding or not organic matter and/or ferric hydroxides - known for the complexation of heavy metals), and spiked them with different levels of copper. The bioavailability of copper was assessed using the passive sampling technique DGT (diffusive gradient in thin film), which provides information on the potential mobility of metals in the sediment and which is supposed to mimic the bioavailable fraction of metals. In parallel, the biological response of the aquatic microphytobenthos to the copper concentration in the exposure media (bioaccumulation and growth inhibition) was analysed. Finally the kinetic of copper uptake by the roots was quantified by the use of a numerical mechanistic model based on the Barber-Cushman approach (plant uptake model).

In addition, the processes influencing the bioavailability of metals (sediment composition, physico-chemical parameters) was assessed by using a probabilistic model (DGT-PROFS) to interpret DGT measurements in sediments. Finally the understanding of the processes of absorption of metals by the root was attempted by comparing experimental results and modelling results (plant model uptake).

WE 274

Chronic toxicity and bioaccumulation in the aquatic oligochaete Tubifex tubifex exposed to Cu-spiked sediment: assessing critical body residue

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Zinc (Zn) and copper (Cu) are heavy metals widely found in the environment. A 14-day exposure experiment under laboratory conditions was conducted to assess the uptake rate constants (k) compared to those measured in worms exposed to natural sediment. Interestingly, at concentrations below the lethality points, at least in an acute exposure. In a chronic exposure, artificial sediments exhibited lower values for reproduction and growth, compared to those measured in worms exposed to natural sediment. Interestingly, Tubifex tubifex Cu in a concentration-dependent manner, both in artificial and natural Cu-spiked sediments. In both experiments, accumulation patterns were almost similar in both experiments, and reproduction impairment was observed at about the same concentration levels. CBR, ranging 3.87 to 4.70 μmol Cu g−1 dw in natural sediment, and 4.18 to 4.66 μmol Cu g−1 dw in artificial sediment. Results showed that copper is a highly toxic metal, with a very narrow range of action, that is effective at low levels of metal tissue concentration. Finally, the ecological relevance of copper CBR levels are discussed.

WE 275

Organotropism and toxicokinetics in tilapia Oreochromis mossambicus during acute/chronic pulsed waterborne copper exposures

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Laboratory toxicity tests are focused on few organs accumulation and performed with constant exposure scenarios to study vulnerability of fish. In reality, pulsed exposure is ubiquitous in aquatic environments. Moreover, the organ-specific copper (Cu) accumulations in fish are critical indicators of Cu exposure. The purposes of this study were conducting acute and chronic pulsed exposure bioassays to assess toxicokinetics of ten organs and tissues based on Cu burden. The Cu distribution dynamics in tilapia Oreochromis mossambicus were also examined. The experimental results found that muscle plays the main distribution dynamics of Cu burden (34.63-50.38%) in the initial 14 days. Moreover, liver is the most accumulated organ (18.77-64.83%) during the exposure periods. The majority of organs in response to acute pulsed exposure showed higher bioconcentration factor (BCF) than those of chronic pulsed exposure, except blood, muscle, and carcass. The gradients of Cu-BCF for ten tissues of tilapia revealed the following pattern: liver > intestine > kidney > stomach > gills > bone > muscle > carcass for acute pulsed exposure, whereas liver > kidney > stomach > intestine > gills > bone > muscle > carcass for chronic pulsed exposure. Here we also investigated the correlations between Cu burden of the fish organs in the acute and chronic pulsed exposures, respectively. The results indicated that the positive correlation between Cu burdens of whole body and liver in acute (r=0.748) and chronic exposures (r=0.607). Obviously, liver plays the most important role in storing Cu concentration, leading to trigger protection action in the pulsed Cu exposure. This work provides the organ-specific distribution dynamics of Cu burden and Cu toxicokinetics information to further develop the physiologically based toxicokinetic and toxicodynamic models. Validation with the experimental results will refine the physiological parameters for pulsed Cu exposure in tilapia was also implicated.

WE 276

Uptake, deparation and bioconcentration of arsenic, zinc and copper mixtures in milkfish (Chanos chanos)

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For As, Zn and Cu in milkfish exposed to single pollutant were 1158.19 ml g−1, following exposure to a mixture containing arsenic (As), zinc (Zn) and copper (Cu). A 14-day exposure experiment under laboratory conditions was conducted to assess the uptake rate constants (k) and deparation rate constants (k−1) as well as the bioconcentration factor (BCF) of milkfish. The values of k, for As, Zn and Cu in milkfish exposed to single pollutant were 1158.19 ml g−1, 851.66 ml g−1 and 13.08 ml g−1, respectively, while the values of k−1 were 62.0 d−1, 2.84 d−1 and 1.24 d−1, respectively. The values of BCF of As, Zn and Cu were 186.71, 299.39 and 10.52, respectively. The interactions among the elements and the subsequent uptake and deparation rates associated with the individual elements were analysed. The results demonstrated that Zn can reduce the accumulation of As and Cu because the high Zn accumulation ability of milkfish. The competition of Zn toward As and Cu may cause the reduction of the growth and the toxicity of the later toxins. Since Cu will enhance the metabolism of the fish, it might be the reason that milkfish will accumulate more As and Zn, when Cu was added into the stock.

WE 277

Combined effect of uranium exposure and phosphorus deficiency on root exudation, uranium bioaccumulation and toxicity in white lupin (Lupinus albus cv. amiga)

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Because of the widespread use that is made of uranium in industry, its concentrations in our environment can locally be higher than background noise. As a heavy metal, uranium is considered to be of toxicological relevance for human health. Plant species represent a major way of transfer of uranium from soils to soils to food chain, but the element physico-chemical properties and speciation in soils makes estimations difficult.

The main goal is the research of physicochemical processes, in particular those linked with plant nutrition and physiology, and their role in defining uranium phytoavailability in the rhizosphere, but also their impact on the bioavailable fraction of metals. In particular, we assumed that there is strong impact of the uptake of uranium in the rhizosphere, with a high concentration in the xylem of the lupin plants. Due to its role in phosphate and ferric nutrition and its chelating properties towards metals, we assume that its presence may have back effects on U solid speciation, U desorption from the solid phases, U uptake and U bioaccumulation in plant species. Because of its proteolytic activity, the white lupin has the capacity to exudate huge amounts of this molecule during phosphorus deficiency periods that makes it one of the key players in these processes.

The work is divided in two parts. In a first one, in hydroponics, citrate exudation by white lupin in different phosphorus and uranium conditions was characterized, as well as toxic effects on lupin development and U localisation in roots. The obtained (and under acquisition) results to be presented will focus in particular on that part, which will then be used to support the in vivo experiments. In this one, phytoavailability of uranium in the simulated rhizospheric effect by adding different amount of citrate (lupin-like amounts) will be studied. For this purpose, we will use RHIZOtest© device and study two contaminated soils where uranium has different origin: a mine tailing and a mine site. For this purpose we will use RHIZOtest© device and study two contaminated soils where uranium has different origin: a mine tailing and a mine site. For this purpose we will use RHIZOtest© device and study two contaminated soils where uranium has different origin: a mine tailing and a mine site.

WE 278

Speciation, bioavailability and toxicity of uranium in different Lemna minor growth media

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ACKNOWLEDGMENTS

The speciation, bioavailability, and toxicity of uranium in different Lemna growth media was valuable. Three different growth media were selected based on their previous use in Lemna growth inhibition tests: (i) the OECD medium [1], (ii) the K-medium [2], and (iii) a synthetic freshwater [3]. For each of the media the pH, CO3 and phosphate concentrations were varied. As expected the OECD medium with normal phosphate concentrations (13.4 mg/L) sustained growth (figure 1A). Changing the pH (figure 1A) or omitting CO3 from the medium did not affect any of the tested growth parameters. However, lowering the phosphate level in the OECD medium clearly negatively influenced growth rate. In contrast for the K-medium it was shown that lowering the phosphate concentration did not adversely influence the growth rate (figure 1B). Only when no phosphate was added the growth rate was below 0.250 average and as such also below OECD guidelines. Finally none of the tested synthetic freshwater compositions could sufficiently sustain growth of the Lemna plants (average growth rate < 0.15/day).

Towards the bioavailability of U only in the K-medium with phosphate concentrations of 0.5mg/L or below U in solution could be retrieved. A dose-response curve for U was
consumption of fish. The area also play an important role. Results obtained from experiments with reconstituted sediment were contrasted with observations from a study with U-spiked field sediment. We determined, in a series of sediment bioavailability/toxicity tests, that different clay minerals with different surface characteristics significantly influence the bioavailability of sediment-associated U and we have quantified binding coefficients. Particle size and associated differences in surface area also play an important role. Results obtained from experiments with reconstituted sediment were contrasted with observations from a study with U-spiked field sediment. All water (overying and pore water), whole-sediment and C. dilutus tissue samples were analysed for U using ICP-MS and the most probable exposure pathway(s) identified. Knowledge of the different U sorption properties of different sediment phases and components will improve local risk assessment of U contaminated sites, and ideally influence the development of site-specific sediment quality guidelines.

WE 280 Mercury concentrations in nesting eagle owl and their main prey, European rabbit, in Southeastern Spain
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Mercury (Hg) is a toxic and nonessential heavy metal of special concern because it is bioaccumulated and biomagnified through the food chain and it is associated with negative effects. Little is known about the bioaccumulation potential of Hg for terrestrial food chains and it is necessary new information about Hg levels in terrestrial species. The aims of this study were to measure Hg concentration in eagle owl chicks from 2006 to 2011 in Southeastern Spain (Murcia) can be considered low (18.10-60.45 µg/g), and it is unlikely that Hg pollution can negatively affect the breeding performance. Significant higher mercury concentrations were found in feathers than in blood (p<0.001). Back feathers could be used as biomonitoring tool for mercury contamination in this terrestrial bird species. We have estimated by simple linear regression the equation: Log Hg in blood (µg/L, ww) = -0.255 + 0.617 * Log Hg in feathers (µg/Kg). Significant positive correlations (r = 0.38-0.61, p < 0.029) were found between Hg concentrations in blood of chicks and levels found in muscle of European rabbit (12.47-15.04 µg/kg ww), their main prey, showing that Hg levels in blood are largely due to current Hg ingested in food collected and fed by the parents. Dietary changes, with a higher proportion of invertebrate and omnivore prey, could be associated with higher mercury levels levels in tissues of eagle owl. Although the studied region is not considered Hg polluted, area under mining influence and the industrial zone seems to contribute in the higher mercury levels in eagle owls and rabbits from the study area and meats from the north region of Spain.

WE 281 Mercury levels in human scalp and fish consumption near the Mid-Atlantic Ridge (Azores)
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The Mid-Atlantic Ridge (MAR) is a geologically active area. Sediments from the MAR ridge near the triple junction of Eurasian, African and North American plates are characterized by a highly toxic environment. The hydrothermal fluids are especially rich in sulphides and heavy metals including mercury (Hg). Mercury uptake by marine organisms is a cumulative process resulting in bioaccumulation and biomagnifications in the food webs, predators accumulate higher tissue concentrations than their prey, putting at risk human health. Located near the triple junction in the North Atlantic Ocean and in the path of the Gulf Stream, the Azores in the MAR and sediments are distributed in both sites of the MAR where hydrothermal fluids biologically affect surrounding ocean waters and potentially inducing different contamination levels in local organisms resulting from its location in relation to the MAR.

Fish usage is commonly used to determine the route of mercury contamination in humans, each Azorean consume on average 76.35 kg of fish per year, making the Azores a Portuguese region with the higher rate of per capita consumption of fishery products. In this study, sediment and human hair were collected from the both sides of the Mid-Atlantic ridge, in the west side, on the Flores island with an annual consumption of 51.2 kg and the east side, on the Faial island with a consumption of 77.3 kg per year and quantified the mercury. The concentrations of total mercury are linked with the weekly consumption of fish.

Biased contamination sources are discussed and the results show a pattern of increased mercury concentration with increasing consumption of fish, other than the normalization of other parameters.

WE 282 Uptake, accumulation and toxicity of arsenic in arsene-tolerant and non-tolerant Agrostis castellana L.
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The tailings of Vale das Gatas mine (Northern Portugal) contain high concentrations of arsenic (540.7-5,770 mg/kg) and heavy metals such as lead and zinc, thus colonizing plants, like Agrostis castellana L., may have developed resistance to these elements. The average values of arsenic accumulated in samples of Agrostis castellana L. ranges from 13.21 mg/kg in the stems to 30.93 mg/kg in leaves.

Due the great chemical similarity, phosphate and arsenate can compete either for the same adsorption sites on soil particles, either by the same mechanisms of absorption by the roots. Thus, it is a serious threat to the centre by the same mechanism of absorption of phosphate. In this study, plants of Agrostis castellana, tolerant and sensitive to arsenate, were tested in relation to As(V) ion as a possible agent of damage to level of root membranes in vivo. Thus, tolerance to As(V) ion was tested by applying a 'root growth test' on specimens from the tailings, with reference to a sensitive population, whose specimens were collected at a location away from the mine. The tolerance index is the ratio of the average ‘maximum roots growth’ in the presence of As(V) and the average ‘maximum roots growth’ in the absence of As(V) to several arsenic concentrations (10, 20, 40, 80, 160, 320, 640, 1280 µM). The results allow verifying tolerance indices significantly higher in specimens that grew up in tailings (59.3% maximum), compared with specimens from remote and not contaminated areas (29.5% maximum).

WE 283 Arsenic and heavy metals in soil and vegetation of a contaminated region
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In this work, it has characterized the species present in the old mining area of Cavaio (Oliveiros, Castelo Branco, Central Portugal); environment with important W, As and some Cs contamination.

The species were selected envisaging their use as bioindicators, meaning that they are able to detect the pollution present in soils for use in mineral exploration; and seeking their potential use in the restoration with metalotolerant species able to tolerate the geochemical stress imposed by these conditions. The analyzed species included: Erica australis, Erica umbellata, Pterospartum tridentatum, Helianthemum nummularium, Helianthemum nummularium, Helianthemum nummularium for Cu; Erica umbellata, Arbutus unedo (leaves), Helianthemum nummularium for Cu; Erica umbellata, Arbutus unedo (leaves and stems), Erica umbellata, Pterospartum tridentatum, Helianthemum nummularium for Cu; and Helianthemum nummularium for Cu.

The results allow verifying tolerance indices significantly higher in specimens that grew up in tailings (59.3% maximum), compared with specimens from remote and not contaminated areas (29.5% maximum).

WE 284 Bioaccumulation of As, Cd, Hg, Pb and Se in the benthic and pelagic food chain of Lake Baikal
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Lake Baikal, the largest freshwater lake and the centre of a vast mountain region. It is the deepest, the most voluminous and the oldest lake in the world. Arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and selenium (Se) were analyzed in water, plankton, invertebrates and fish samples from Lake Baikal with high resolution inductively
coupled plasma mass spectrometry (HR-ICP-MS). The present study confirms generally low concentrations of potentially toxic chemical elements (As, Cd, Hg, Pb) and Se in Baikal Lake in comparison to other freshwater ecosystems. However, the comparative study of water and benthic biotypes in the ecosystem of Lake Baikal revealed that bioaccumulation potential and distribution of the chemical elements differ between locations with riverine and possible anthropogenic influence. The problem is that the seagulls are highly higher in water samples from the Selenga Shallows in comparison to Listvyanka Bay, while concentrations of Pb were significantly higher in fish from Listvyanka Bay. This may be related to greater bioavailability of Pb for fish at Listvyanka Bay caused by lower abundance of natural ligands at this location. Similar phenomenon was observed for Cd, Hg and Se were higher concentrations were found in plankton at Listvyanka Bay. Nevertheless, lack of difference between concentrations in water. In both the pelagic and benthic food chain of Lake Baikal, Hg showed indications of biomagnification while As, Cd and Pb showed indications of biodilution. When comparing fish inhabiting the pelagic and benthic food chain of Lake Baikal, differences in concentrations of As and Hg were found. Arsenic concentrations were twice as high in pelagic fish, while the benthic fish had seven times higher Hg content compared to pelagic fish. The Se:Hg molar ratios revealed that although Baikal is located in a Se deficient region, Se is still in significant excess over Hg. Based on this relationship there is probably no risk of Hg induced toxicity in the endemic fish species of Lake Baikal at the present.

WE 285
The bio-accumulation of selected metals (Cu, Fe, and Mn) in water, sediments and invertebrates (Physa acuta) of Kuilsriver, Western Cape, South Africa
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In this study, metal analysis of water, sediments and freshwater snails from Kuilsriver in Western Cape, South Africa, were carried out with a vision to attain a baseline data of their presence and also levels found to pose the threat to Kuils River. Samples were collected randomly every two months from selected sites of the Kuilsriver for a period of one year. Samples were digested with 55 % nitric acid. The Inductively Coupled Plasma - Atomic Emission Spectrometer (ICP-AES) were used for metals (Cu, Fe and Mn) analysis. The concentrations of ranges of all the metals measured were found to be below the international guidelines for Sediments. South African guidelines were used for water comparisons. Then, literature reviews for snails were used. All comparisons done for water and sediment showed kuilsriver to have very low detected metal concentrations; which can be considered as acceptable. However, for Snails these metals are micro nutrients, it was complicated to conclude whether concentrations were toxic or normal to the species.

WE 286
Assessing the combinatorial toxicology of the anthropogenic challenges associated with the marine environment in the proximity of Jeddah city in the Red Sea
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The coastal marine environment of the Red Sea is subject to direct and indirect influences of major populations and industrial facilities found along the coast such as those discovered in proximity to Jeddah City. When evaluating ecological impact of diffuse pollution from urban environments, it is important to consider interactions between contaminant mixtures that individually trigger the health and environmental consequences. To determine whether mixture effects need to be considered, it is essential to define geochemical parameters by performing a survey for major classes of contaminants and to evaluate penetration into the food chain. Sampling of both sediment and seawater at contrasting sites representative of near-shore (25m) vs. off-shore (10km) locations. Possible food chain transference of any contaminant was evaluated by sampling fish (L. nebulosus) and plankton at the off-shore sites. All samples were assessed for the presence of heavy metals (ICP-MS) and hydrocarbons (GC-MS). Results are achieved our objective of defining the contaminant profiles within the water columns and transference to the biota. The elevated metals in the off-shore samples were intriguing and may be related to the deep-water brines previously identified in Atlantis II at the axial depression of the Red Sea. Furthermore, uptake of Al from the sediment to the gills and livers of L. nebulosus indicated the role of sediment as a source for heavy metal uptake into the food chain. Having identified the major inorganics acting as toxicants within the interface between Jeddah City and the Red Sea and recognising the geogetic input from the deep-water brines, we are in a position to test for consensus among mixture effects using the model organism, C. elegans.

WE 287
Dissolved selenium speciation (selenite versus selenate) affects uptake and biotransformation in freshwater periphyton
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The aim of this study was to evaluate concentrations of heavy metals and other trace elements in the aquatic macrophyte Potamogeton pusillus, matching this information with the corresponding to water and sediment samples from the Suquía River basin. So far, we looked to assess whether this aquatic macrophyte reflects different pollution scenarios in both shell and soft tissues, and if the heavy metals concentrations are analytical determined and subsequently compared to highlight intrinsic correlations and develop a possible explanation of metal partitioning. In addition it was determined the different species responses to bioaccumulation tests to achieve an indication about their ability to accumulate the selected toxic metal in its shell. This skill could be used in future applications concerning the comprehension and the following exploitation of Bivalves role in water basin self-purification mechanisms.

WE 288
Bioaccumulation of heavy metal and trace elements by Potamogeton pusillus as bioindicator of aquatic pollution
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Potamogeton pusillus is a native species widely distributed throughout the basin, which was selected considering its favorable characteristics for use as a bioindicator (big quantity of plants, easy harvest and maintain in laboratory). Plants were collected during the wet season at two stations along the Suquía River basin, having different pollution degrees and anthropogenic impacts. Potamogeton pusillus is a native species widely distributed throughout the basin, which was selected considering its favorable characteristics for use as a bioindicator (big quantity of plants, easy harvest and maintain in laboratory). Potamogeton pusillus is a native species widely distributed throughout the basin, which was selected considering its favorable characteristics for use as a bioindicator (big quantity of plants, easy harvest and maintain in laboratory). Potamogeton pusillus is a native species widely distributed throughout the basin, which was selected considering its favorable characteristics for use as a bioindicator (big quantity of plants, easy harvest and maintain in laboratory). Potamogeton pusillus (hereinafter referred to as P. pusillus) is an ecologically and commercially important macrophyte species of soft sediment inter-tidal communities throughout the northern hemisphere and is known to be impacted by various pollutants. More specifically in many coastal locations, N. viripes is exposed to a range of heavy metals including copper and zinc which are known to be highly toxic to periphytons. This species is also collected for bate which affects their abundance and may change the bioavailability of these metals in the sediment, and those available to the worms. To assess the relationship between copper and zinc concentrations in the sediment, pore-water and associated worms, samples will be collected from a number of sites in the UK and sites adjoining the English Channel, with different pollution histories and various levels of bai
chicks may not be an appropriate proxy for adults when using stable isotopes. Also, the POPs did not have a uniform placement in the PCA plot of both species and tissues. Additionally, the species results for trophic level were in contrast to previous studies of adult birds. The individuals used in the present study were chicks, which suggest that...

In the present study, muscle samples from three seabird species (Rissa tridyctalis, Fulmar glacialis, and Larus argentatus) were used to investigate these issues. The birds were...

regression, also known as the trophic magnification factor (TMF).

However, seabird species differ in physiology and ecology; even though species may have the same trophic position, they may have different concentrations of POPs, or vice versa.

Seabirds are top predators in aquatic food webs, and exposed to persistent organic pollutants (POPs) primarily through diet. POPs undergo trophic transfer and some biomagnify within the tissues to higher trophic levels. There is thus a great substantial need to understand the bioaccumulation and toxicity of metals in aquatic organisms, since it can provide critical information for the environmental risk assessment of metals in aquatic environments.

The present study considered the bioaccumulation of metals such as cadmium (Cd), lead (Pb) and zinc (Zn), including precious metals such as platinum (Pt), palladium (Pd) and rhodium (Rh) in sediment and freshwater crabs from sampling sites in close proximity to precious metal mining activities in the North-West Province, South Africa.

The results showed contamination of the water bodies and bioaccumulation of heavy metals in prawns. This portends a serious public health risk. Consequently, continuous water body pollution monitoring needs to be embarked upon to ensure clean water bodies.

The populations of mollusks and crustaceans in the Epe Lagoon are threatened by pollution, which is causing a decrease in their populations. This is particularly evident in the case of prawns and other crustaceans such as crabs, which are important food sources for many aquatic organisms and humans.

The results of this study are important for understanding the impact of pollution on the aquatic ecosystem and the health of local residents who depend on these resources. Moreover, the results can inform policies and interventions aimed at mitigating pollution and protecting aquatic ecosystems.
samples were collected from 8 farms (4 samples from each farm and 2 farms from each station). 4 water samples were collected. Biometric were performed, then 10 grams of water were collected over large geographic areas along the Pacific coast of North and South America, China, Japan and Hong Kong. Using aquatic ecosystems via aerial drifts, runoffs and leaching where it affects habitants of aquatic ecosystems. A field simulated study was carried out to assess the effects of pirimiphos methyl in the Matopos dam water, carboxylesterase activity was inhibited in the range 45-72% in L. natalensis compared to inhibition in the range 58-88% in H. duryi. LT50 determined in L. natalensis, natiness and up to 76% in H. duryi depending on exposure period. Our results showed that pirimiphos methyl definitely affected the well being of the aquatic snails and this has a negative effect on the entire aquatic ecosystem as snails have a role in maintaining a healthy aquatic ecosystem. They are prey for other organisms such as some fish and frogs. It is also feeding on a variety of substances, contributing to waste removal and helping them in maintaining a balanced aquatic ecosystem.
WE 305
Bacteria associated with skeletal tissue growth anomalies in the coral Platygrya carnosus
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Skeletal growth anomalies, often referred to as “tumors”, have been reported globally. A recent survey of Hong Kong waters showed that more than 60% of Platygrya carnosus colonies developed tumors. Here we report for the first time, the bacterial community associated with tumors in P. carnosus over different seasons and locations in Hong Kong. Culture-based methods for strain isolation and molecular techniques of 16S rRNA analysis for strain identification were used. The culture-independent technique terminal restriction fragment length polymorphism would be considerably different between healthy and tumor corals and aimed to validate that potential differences because of tumors would overcome the seasonal and spatial influences. Our analysis detected only minor differences between the communities associated with the healthy and tumor corals, indicating that tumors are not associated with major microbial community structures. In contrast, community structure was strongly influenced by season and location. This study is related to environmental variables (i.e. season and location) than to disease (i.e. tumor).

WE 306
Identification of alternate solutions to mitigate river health degradation and their effect on ecosystem services in low flow river watershed
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Global change impacts on water availability, water quality and ecosystem services in Mediterranean river basins of the Iberian Peninsula, as well as their impacts on the human society and economy makes it a key issue on the EU agenda. It is likely that the first impacts of climate change will be felt in the Mediterranean water resource system through increased frequency of water shortages and decline in water quality. Excessive nutrient loading from agriculture, industrial and domestic sectors is expected to become a growing threat to low flow river Frankol, located in Catalonia (Northeastern Spain). Water quality degradation is getting severe in areas where water is scarce due to reduced capacity of water management and the necessity for new water resources in the low flow river watershed. Some alternate solutions to the increase of water demand may include desalination, inter basin transfers, using ground waters and ground water aquifer recharge. In parallel to technical solutions, an attractive aspect for river basin managers is the promotion of responsible water use in the basin. To evaluate and improve water management in the Mediterranean, two steps are followed. First, different hydrological scenarios are simulated using MIKE BASIN software. Then, spatially explicit modelling tool, called Integrated Valuation of Ecosystem Services and Tradeoffs (InVEST) is used to assess the ecosystem services of different modelled hydrological scenarios. The evaluated services are water yield and supply, nutrient and sediment retention. Ecosystem based approach to river basin management, that consider the trade-offs between different inland water ecosystem services are more likely to ensure sustainable solutions. The results of this work are expected to improve the understanding of the management problem, and help to identify the best management solutions for the studied basin.

WE 307
Tailoring of InVEST model to assess climate change impacts on ecosystem services at a Mediterranean river basin
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The Mediterranean basin is considered one of the most vulnerable regions of the world to climate change and with a high potential to present important water problems in the near future. The predicted future scenarios for this region present an increased frequency of floods and extended droughts, especially at the Iberian Peninsula. These events are likely to produce important changes in the ecosystem services provided by Mediterranean river basins.

To evaluate the impacts of climate change on ecosystems services of Mediterranean river basins, we apply InVEST, a spatially explicit tool consisting of a suite of models that use land use and land cover patterns to estimate ecosystem services from a biophysical and economic viewpoint. This tool was applied to evaluate and quantify water supply, nutrient and sediment retention and erosion protection services. For the application of the tool for model and the inclusion of some peculiarities of the studied region were made. These changes regard the incorporation of water extraction and return points, and the possibility of valuing water for uses other than the hydropower production. These improvements are key for the model application to the studied areas, since they strongly influence the dynamics of Mediterranean basins.

To evaluate the appropriateness those improvements, the modified tool was applied to the Llobregat river basin, located at Catalonia (NE of Spain). The Llobregat River is the second longest river in Catalonia, and one of Barcelona’s major drinking water supplier. This catchment receives extensive urban and industrial wastewater discharges as well as surface runoff from agricultural areas. The results of this study will be validated with real data from the studied basin.

WE 308
Testing strategies in Ecotoxicology: two examples for non target arthropod testing from China
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As an example of this approach, two terrestrial ecotoxicology test systems are presented, both standardized for Chinese registration processes and approved by the national Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA). Setup, handling and results of terrestrial toxicity assessment of Trichogramma (most frequently used species: T. caecanemus, T. evanesens) and the silkworm Bombyx mori are presented, both species required by ICAMA and described in Chinese guidelines for ecotoxicological testing.

Trichogramma has a worldwide geographical distribution and plays an important role as natural enemy against lepidopterous pests on a wide range of agricultural crops. It is regarded as a sensitive indicator species, suitable for identifying plant protection products that are unlikely to be harmful to non-target arthropods in the field. The testing guidelines of ICAMA (2004) and IOBC (2000) differ strongly considering the duration of the exposure phase and the type of test cages.

Bombyx mori is also widespread in China, Cambodia, Vietnam, Japan, India, Southern Europe and especially Brazil. Due to their economic importance and sensitivity to xenobiotics, larvae of B. mori are regarded as suitable for hazard assessment of plant protection products and biocides.

Both examples will demonstrate that the registration of plant protection may depend on regional requirements either by using region-specific testing procedures (see Trichogramma) or even region-specific test species (see Bombyx). The differences in handling, required test species, regional experiences as well as cultural aspects show the difficulties that can appear within ecotoxicology testing and global registration of plant protection products.

WE 309
Selenium cycling in an estuarine ecosystem receiving inputs from coal-fired power station
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Selenium (Se) concentrations in the water column of Lake Macquarie in New South Wales are elevated in comparison with other estuarine systems. Selenium is regarded as a potentially toxic element of concern mainly due to its bioaccumulation in aquatic food webs. Selenium bioaccumulation and biomagnification in the seagrass food webs were examined by using C and N isotopes to establish food chains. As well the volatilization of selenium from sediments was measured. This work clarifies the current and historical inputs of selenium and characterizes how selenium may be lost from sediments by volatilization or remobilization into marine food webs.

WE 310
Adaptation strategies in ecological and ecosystem services: valued mercury contamination
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The Amazon rainforest provides many ecosystem services that are important locally, regionally and internationally. These ecosystem services represent the good and processes that are important for human well-being, obtained directly or indirectly from ecosystems. A service that is little known or considered is the forest provides goods and services against pollution of aquatic ecosystems by mercury. Much of the mercury in the Amazon is stored naturally in the soil. With the removal of vegetation mercury is transported to aquatic ecosystems enabling its transformation into methylmercury. This substance has, among other mercury species, the greatest potential for...
bioaccumulation and biomagnification in different trophic levels, starting by aquatic organisms. This study was conducted using the Purus River Basin as a model to simulate the damage promoted by the input of mercury in aquatic systems produced by deforestation, and the valuation of those damages. Data of soil types, concentration of mercury in soils, methylation rate and concentration of MeHg in fish were collected from literature. Hence, the model estimated the potential costs of mercury poisoning treatment, as a consequence of forest clearing, by using a function with the following variables: the deforestation, the concentration of mercury in the soil, the rate amount of soil eroded, the level of mercury methylation, MeHg concentrations in fish, fish consumption by humans, damage to health and costs expended in diagnosis and treatment of poisoning. Assuming a deforestation rate of 8% of the basin per year, which is the current rate of deforestation, the potential damage costs would be approximately $1 million/year. This estimated cost represents the value of ecosystem service of entrapment of mercury in the soil due to protection provided by the forest against erosion.

WE 311

Hypoxia and ecosystem function


University of Hong Kong, Hong Kong, Hong Kong

The Chinese University of Hong Kong, Hong Kong, Hong Kong

European commission, Marston, Worcestershire, United Kingdom

Abstract

Hypoxia, defined as a partial pressure of oxygen below 4 mg/L, can have severe consequences for the invertebrate community structure. At the same time, some hypoxic biofilms were allowed to recover in normoxia in the laboratory for one week and subsequently, tested for settlement response. A further concept in the assessment of rivers statuses is the comparison to reference sites. According to the level of different variables, river sections are divided into natural, semi-natural and degraded types. This information forms the basis for determining net ecosystem services (benefits and costs) associated with different management scenarios. As ecosystem services are increasing at an alarming rate, especially in urban areas, they are gaining importance for small scale farmers, the input parameters for the index (including social and economic parameters if available) as well as the status of the ecosystem services as a baseline assessment. The input variables for the index are obtained through Participatory Action Learning workshops with the small scale farmers communities.

WE 312

The use of an ecosystem services performance index to describe the status of water quality impacted by small scale farming communities in Limpopo, South Africa

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A village at the selected sites. Water quality parameters measured included nutrients, microbial content, suspended matter and dissolved metal concentrations. Sediment samples were analysed for metals and pesticides. Low WBI scores were obtained in the upper catchment that is subjected to runoff from urban settlements, agriculture and mining activities. As the water flows into the wetland the level of pollutants decrease and this is reflected in the increased WBI scores. During the rainy season the sites upstream of the wetland have much lower WBI scores with concomitant higher microbial, nutrient and metal loads. However the water flow is attenuated by the wetland system resulting in higher WBI scores and lower pollutant concentrations. The highest index scores and best water quality is found at the most downstream site, which is situated in an impoundment that is used to supply potable water to a nearby town. We demonstrated that the WBI is a useful monitoring tool to determine the effectiveness of the pollution control measures. This information forms the basis for determining net ecosystem services (benefits and costs) associated with different management scenarios. As ecosystem services are increasing at an alarming rate, especially in urban areas, they are gaining importance for small scale farmers, the input parameters for the index (including social and economic parameters if available) as well as the status of the ecosystem services as a baseline assessment. The input variables for the index are obtained through Participatory Action Learning workshops with the small scale farmers communities.

WE 313

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Abstract

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WE 314

The application of a wetland biotic index to assess the pollution control function of the Nylsvley wetland, South Africa

R. Greenfield, V. Wepener, J. H. J. Van Vuren

University of Johannesburg, Johannesburg, South Africa

Wetlands form a vitally important part of the river systems around the world. They perform vital functions that help maintain the quality and quantity of fresh water in many regions. One of the essential services that wetlands provide in developing countries is related to pollution control. This provides a natural cleansing function for safe water by downstream users. Bio-assessments are based on the premise that the biotic communities will reflect the health or integrity of the wetland system. The aim of this study was to determine whether the Wetland Biotic Index (WBI), which was developed specifically for riverine floodplain wetlands, was able to demonstrate the pollution control function of the Nylsvley wetland in the Limpopo province of South Africa. The Nyl River and floodplain are subjected to various impacts such as mining and farming as well as runoff from forested and artificial wetlands. Sampling sites were determined along a gradient of change in WBI scores in relation to changes in water quality, as the Nyl River moves through the wetland. The WBI index consists of three metrics, i.e. a macroinvertebrate index based on sensitivity scores, a habitat quality and land-use rating. The WBI scores were determined on a seasonal basis from six sites in the Nylsvley system. Concurrent water and sediment quality analyses were undertaken at the same sites. Sediment quality parameters, microbial content, suspended matter and dissolved metal concentrations. Sediment samples were analysed for metals and pesticides. Low WBI scores were obtained in the upper catchment that is subjected to runoff from urban settlements, agriculture and mining activities. As the water flows into the wetland the level of pollutants decrease and this is reflected in the increased WBI scores. During the rainy season the sites upstream of the wetland have much lower WBI scores with concomitant higher microbial, nutrient and metal loads. However the water flow is attenuated by the wetland system resulting in higher WBI scores and lower pollutant concentrations. The highest index scores and best water quality is found at the most downstream site, which is situated in an impoundment that is used to supply potable water to a nearby town. We demonstrated that the WBI is a useful monitoring tool to determine the effectiveness of the pollution control function of ephemeral floodplain wetlands.
subsequent adjustment.

In this case study, a net ecosystem services analysis (NESA) was applied in support of the re-registration of a well-known insecticide used in Southern European countries.

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3) Comparative genomic analysis to identify commonalities in stress response pathways between Eisenia and Folsomia when exposed to the same stress factor.

2) Gene expression analysis that reflect negative effects of allyl ITC on soil invertebrates, using microarrays.

and liver were analyzed and the resulting biological effects were determined by a combination of analysis of gene expression and micronuclei formation. Twelve biomarker relationships were observed at the highest U concentration (1.0 mg/L). All biomarker genes tested were found to be up-regulated by DU. Microarray analysis clearly showed that the number of differentially expressed genes as well as the biological processes significantly regulated by U also appeared to be concentration-dependent. Micronuclei assay on blood cells showed an apparent, albeit non-significant increase of micronuclei formation at 0.25 mg U/L. The results suggest that DU may have concentration-dependent mechanisms of toxicity in Atlantic salmon, giving rise to the regulation of different biological processes as well as the potential induction of several toxicological pathways, which commonly were not found to lead to immediate detrimental effects (e.g. micronuclei formation), potentially due to the short exposure duration in this study.

WE 323

Toxicogenomics - a tool for the assessment of sediment quality.


German Federal Institute of Hydrology, Koblenz, Germany.

The analysis of gene expression in the context of ecotoxicology is of growing interest, because in principle it allows the simultaneous analysis of exposure and effect and can thus help to answer the question if a contamination might be a pollution or not. This question has high relevance for the risk assessment of contaminated river sediments. The sediment is an ecosystem (benthos) with organisms reacting to particle bound pollution. Sediments can act as both, potential sinks but under other conditions as potential sources for many hazardous substances. Therefore, several methods for sediment contact tests with various samples were developed in the recent years. In the present study sediment contact tests. Data from (Oryza sativa) were performed. Oryza sativa can be grown under natural conditions and has been at a prerequisite for a comprehensive expression analysis of a sequenced genome. In order to initially characterize macroscopic effects of Ni on rice development (Oryza sativa), the ground rice grains were exposed on OECD sediment 218 spiked with NiCl2 at various concentrations. The most sensitive end point for exposure to Ni was the root length; in addition to growth inhibition a different morphologies of the root was observable. Roots of exposed plants were less branched and thicker compared to the unexposed control. For the analysis of gene expression the custom 60k salmonid oligonucleotide microarray was used. Bioinformatic analysis of the expressed genes showed that the expression of several genes is associated with a different response of Oryza sativa to Ni. The results showed that Ni can cause a different effects on the expression of genes associated with the root development.

WE 324

Natural toxins and their molecular and life-history effects on non-target soil invertebrates.

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VU University Amsterdam, Amsterdam, Nederland

The effects of endocrine disruptors on non-target organisms, such as soil invertebrates, are becoming increasingly important in ecotoxicology. Many xenobiotics have been shown to be toxic to non-target soil organisms and could thus affect the functioning of the soil ecosystem. Microarrays have been used to study the effect of endocrine disruptors on soil invertebrates. However, the results have been inconsistent and the understanding of the mechanisms behind them is still limited. In this study, we used microarrays to investigate the effects of two endocrine disruptors, DDT and PAH, on the expression of genes in the nematode Caenorhabditis elegans. The results showed that DDT and PAH had different effects on the expression of genes in C. elegans, with DDT affecting the expression of genes involved in the stress response and PAH affecting the expression of genes involved in the immune response. This suggests that the effects of endocrine disruptors on non-target organisms are complex and depend on the specific compound and the target organism.

WE 325

Establishment and application of Chinese Rare Minnow (Gobioocypris Rarus) endocrine system functional genome cDNA microarrays.


Research Center for Environmental Sciences, Chinese Academy of Sciences, Beijing, China.

Functional Genomics will be a powerful tool to exhibit the function of endocrine system tests in fish. Unfortunately, there are relatively few functional genes in GenBank which limits the use of molecules to study disruption of endocrine responses in fish. In this research, we will select Chinese rare minnow as a model fish and develop functional genes in the juvenile Atlantic salmon (Salmo salar). The protein-protein-protocatechuic acid (PPOX) in fish gene clone and R1PCR methodology. Molar targets of the three endocrine axis will be confirmed after exposure to a certain endocrine disruptor, and molecular toxicological mechanism of endocrine disrupter will be elucidated. Endocrine system functional gene cDNA microarrays of Chinese rare minnow were prepared. The feasibility of cDNA microarray was evaluated and the applicability of this technology in environmental risk assessment was confirmed.

WE 326

Toxicogenomic analysis of benzo[a]pyrene, toluene and formaldehyde on Drosophila melanogaster and Caenorhabditis elegans.

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University of Seoul, Seoul, South-Korea (Rep)

The effects of benzo[a]pyrene, toluene, and formaldehyde on the expression of genes in the nematode Caenorhabditis elegans were investigated. The results showed that exposure to benzo[a]pyrene, toluene, and formaldehyde caused a significant decrease in the expression of genes related to stress response and immune function. Furthermore, the results revealed that the effects of these compounds were not additive but rather synergistic, which highlights the importance of considering the combined effects of multiple pollutants. It is hoped that these findings will contribute to the development of new strategies for the prevention and management of environmental health risks associated with exposure to these compounds.
The results showed that EF1a had no significant variation in expression among the three different tissues. This expression remained stable between control and short plants. This clearly indicated that early life stages of fish development were sensitive to low concentrations of EDC, and such exposure is now known to lead to distinctive physiological impairments caused by long-term exposures to various chemicals. In this study, we investigated long-term effects of sex steroids on gene regulation between target site resistant plants (GMO) and susceptible (WT) plants. Annotation of these genes is currently underway using publicly available Z. mays genomic information and will provide clues to the underlying mechanism involved in the observed expression of dry weight in plants following treatment with 27 g/a glyphosate.

Further research is required to understand the mechanism of physiological impairments caused by long-term exposures to various chemicals. It is well known that Real Time PCR (RT-qPCR) is the preferred method for studying gene expression because of its sensitivity, precision and robustness but it requires the use of reference genes. In this study, we have used the translation elongation factor 1 alpha (EF1a) and Actin (ACT) in B. laevis in three tissues (root, stem and leaves) under four conditions: 1-control-: Large plants (>1500mg), temperature 22ºC, media: Hoagland solution; 2 -short-: Short plants (<250mg), temperature 22ºC, media: Hoagland solution; 3-target site resistant-: Large plants, temperature 22ºC, media: Hoagland solution + endosulfan 10µg/L; 4 -cold-: Large plants, temperature 5ºC, media: Hoagland solution. All plants remained 24hs under each condition with 3 replicates tested for each one. Firstly, the partial sequences for both genes were obtained from degenerate PCR primers. The two sets of sequences showed an optimization of 82% (EF1a) and 81% (ACT) compared to other members of Asteraceae family. From these sequences a set of primers for each gene was designed and the RT-qPCR was optimized.

The results showed that EF1a had no significant variation in expression in the three different tissues. This expression remained stable between control and short plants. However, a significant increase in expression was observed in plants exposed to endosulfan or cold. In contrast, ACT expression did not show significant differences among tissues or under any condition tested in this study. From these results we can conclude that Actin expression did not seem to be influenced in short plants or during endosulfan or cold exposure and can thus be used as a reference gene for RT-qPCR in any tissue, while Elongation Factor-1 alpha (EF1a) should not be used when analysing stress conditions such as cold and xenobiotic exposure.

Defining the transcriptome of the fathead minnow liver using high-throughput sequencing: effects of chemical and environmental stressors
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University of Saskatchewan, Saskatoon, Canada

The fathead minnow (Pimaphales promelas) is a popular small fish model routinely used in regulatory testing and basic research in environmental toxicology. More information about chemical toxicity has been derived from studies with the fathead minnow than any other species of fish. In the past decade, following the advent of high-throughput 'omics technologies, the fathead minnow has been used as a model species for proteomics, metabolomics and transcriptomics. The vast majority of the transcriptomic studies of the fathead minnow have been performed using Illumina RNAseq platforms. However, to date, no studies had been performed by use of recently developed open-format high-throughput transcriptome sequencing technologies such as Illumina RNAseq. As a consequence, large-scale characterization of the transcriptome of the fathead minnow liver had not been performed. We are actively characterizing the transcriptome of the liver of the fathead minnow in order to quantify transcriptional responses to various natural and anthropogenic environmental stressors. In one series of studies we have been exploring the transcriptional responses on fathead minnows acclimated to low temperature. In a second experimental series, we have exposed fathead minnows to glyphosate, a herbicide commonly used in agriculture soils, by the Canadian Environmental Quality Guidelines) and 250 mg/kg soil (5x the previous concentration) and to the pesticide dimethoate at 0.4 mg/kg soil in gene regulation between target site resistant plants (GMO) and susceptible (WT) plants. The fact that the glyphosate resistant Z. mays did not respond to the glyphosate treatment as the WT plants did, shows that the hormetic response is directly related to glyphosate binding to its target site. Microarray analysis of both surfactant (untreated) and glyphosate treated plants showed qualitative differences in gene expression between target site resistant plants (GMO) and susceptible (WT) plants. Annotation of these genes is currently underway using publicly available Z. mays genomic information and will provide clues to the underlying mechanism involved in the observed expression of dry weight in plants following treatment with 27 g/a glyphosate.

The toxicological phenomenon termed hormesis is described as a biphasic dose-response, characterized by low-dose stimulation & high-dose inhibition. Although hormesis has often been observed for the effect of the herbicide glyphosate sprayed on plants at doses of 10-40 g/a-1, the physiological mechanism(s) behind this phenomenon are currently poorly understood. Functional genomic studies and microarrays in particular are very helpful in identifying and characterizing groups of genes that are differentially regulated, in response to a physiological stimulus. The availability of genomic information for Z. mays, as well as the existence of a genetically modified glyphosate-target site resistant mutant (GMO), makes this species ideal for such a study. In this study we present microarray analysis of mRNA isolated from GMO and wild type (WT) Z. mays tissues treated with 27 g/a glyphosate ha-1 compared to plants sprayed with surfactants only. Plant material for mRNA analysis was harvested 24 h after spraying. Plant dry weight 10 days after spraying was (± standard deviation) 0.17 g ± 0.02, 0.19 g ± 0.02, 0.17 g ± 0.02 and 0.24 ± 0.02 for glyphosate treated and un-treated GMO and WT plants. The fact that the glyphosate resistant Z. mays did not respond to the glyphosate treatment as the WT plants did, shows that the hormetic response is directly related to glyphosate binding to its target site. Microarray analysis of both surfactant (untreated) and glyphosate treated plants showed qualitative differences in gene expression between target site resistant plants (GMO) and susceptible (WT) plants. Annotation of these genes is currently underway using publicly available Z. mays genomic information and will provide clues to the underlying mechanism involved in the observed expression of dry weight in plants following treatment with 27 g/a glyphosate.

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Acknowledgement - This work was supported by Mid-career Researcher Program through NRF grant funded by the MEST (2011-0027489).

Hb cDNA of C. riparius (CrHb) were identified by screening through an expressed sequence tags database developed from the fourth instar larvae of Chironomus riparius and contains an open reading frame (ORF) of 504 bp. The deduced CrHb protein has 169 amino acids. Expression pattern of CrHbs were studied using real-time polymerase chain reaction after short term exposure to environmental pollutants and the results suggest that the expression profile of CrHb may potentially contribute to the development of a biomass maker for ecotoxicity monitoring in C. riparius.

Acknowledgement - This work was supported by Mid-career Researcher Program through NRF grant funded by the MEST (2011-0027489).

WE 334

Stress response gene expression in Chironomus riparius exposed to various environmental pollutants

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Aquatic organisms are exposed to various kinds of environmental pollutants having different modes of action released from various anthropogenic sources which can disrupt many biological functions. Recently next generation sequencing (NGS) technology has been increasingly used in wildlife species gene characterization in an attempt to identify their environmental stress response which has high potential for ecological studies. Such stress proteins are characteristic of oxidative stress marker and have been widely used as oxidative stress biomarker in ecotoxicology, whereas, p38MAPK has rarely been approached in an ecotoxicological context, although, the roles of p38MAPK in stress responses have frequently been reported in various biological systems. In this study, we identified and characterized SOD and p38 MAPK in C. riparius, by screening through an expressed sequence tags (EST) database developed from the fourth instar larvae of Chironomus riparius using 454 pyrosequencing. We also investigated the response of the previously characterized stress response genes toward various environmental pollutants and their responses will be compared with higher level effect such as development and reproduction. This comprehensive stress response profile will provide an insight into the stress response mechanism of important sediment toxicity model species, C. riparius and also offers information on the relationship between stress response gene expression and higher level effect.

Acknowledgement - The work was supported by Mid-career Researcher Program through NRF grant funded by the MEST (2011-0027489).

WE 335

Risk assessment of wood smoke particles using a test battery

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Wood burning is a major source of air pollution and is a reason for enhanced hospital admission and mortality. Data on the MOA exhibited by wood smoke particles is insufficient. At present we cannot provide an adequate risk assessment for wood smoke. For this we intend to implement a method. Celereans appears as an optimal surrogate to assess potential environmental impacts for the organism because it is frequently used in ecotoxicological and anthropogenic biochemical studies. Gene expression analysis of selected biomarkers which are involved in supposed mechanisms are used to give insight into the mode of action exhibited by wood smoke particles. Results were obtained by assays with Celereans are complemented by microbial bioassays that are conducted by a colleague. Wood smoke particles were used in an electrostatic precipitator to establish a nematode test adapted to the use with wood smoke particles in suspension. Gene expression analysis was conducted via realtime PCR of 4 previously selected genes that are involved in xenobiotic detoxification, inflammation and oxidative stress.

Contrary to expectations sample B from complete combustion i.e. with low PAH content exhibited higher toxicity than sample A from incomplete combustion and high PAH content. We suspect low biavaliability of PAH from sample A and high availability of zinc from sample B to cause the toxicity levels. The results from gene expression analysis shown in 2 support the argument drawn from the nematode test. Cy335C1 reflecting the bioavailability of PAH is strongly expressed in sample A. Expression differences of Cy335C1 and p38 MAPK do not reflect the expression levels. Sample C can not be completely ruled out, because from sample C we do not observe the expected expression of superoxide dismutase (Sod3) in sample B supposes oxidative stress probably caused by zinc, which is supported by the standard nematode test and a battery of microbial bioassays.

The adaption of the nematode test to wood smoke particles was successfully implemented. The results suggest that chemical analysis and common predictions do not adequately project fine dust toxicity. Further studies on the molecular mechanisms of fine dust toxicity are going on.

WE 336

Effects of munitions compounds and breakdown products on Peroxisomal Proliferator activated receptors Alpha, Beta/Delta, and Gamma

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Background: Peroxisomal Proliferator activated receptors (PPARs) have emerged as key players in cellular processes involved in the regulation of lipid metabolism, inflammation and carcinogenesis. This family of nuclear receptors consists of three subtypes: alpha (PPARα), beta/delta (PPARβ/δ) and gamma (PPARγ). PPARα has been shown to be involved in the regulation of adipogenesis, inflammation and oxidative stress. PPARγ is involved in the regulation of adipogenesis and insulin sensitivity. PPARβ/δ has been shown to regulate cholesterol metabolism and inflammation.

Purpose: The purpose of this study was to investigate the effects of munitions compounds and breakdown products on PPARα, PPARβ/δ, and PPARγ expression in vitro in mouse liver cells (MLC) and in vivo in mice exposed to munitions compounds.

Methods: The in vitro studies were conducted in collaboration with the U.S. Army Environmental Research and Development Agency (ERDA) at Aberdeen Proving Ground, MD. MLC were treated with various concentrations of munitions compounds for 24 hours and cell extracts were used for real-time quantitative PCR analysis of PPARα, PPARβ/δ, and PPARγ. The in vivo studies were conducted at the Marine Corps Base Quantico, VA. Mice were exposed to munitions compounds in their drinking water for 14 days and were sacrificed at day 14. Liver and muscle samples were collected and used for real-time quantitative PCR analysis of PPARα, PPARβ/δ, and PPARγ.

Results: In the in vitro studies, the following compounds were shown to affect PPARα, PPARβ/δ, and PPARγ expression: 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), 2,4,6,10,12’-hexinitro-2,4,6,8,10,12-hexaazaisowurtzitane (CL-20), 1,3,5-trinitrobenzene [1,3,5-TNB], 2,4-dinitroanisole [DNAN], and 5-nitro-2,4,2-triazol-3-one (NTO). These compounds were shown to significantly affect PPARα, PPARβ/δ, and PPARγ expression in a dose-dependent manner. In the in vivo studies, mice exposed to munitions compounds showed a significant increase in PPARα, PPARβ/δ, and PPARγ expression in both liver and muscle tissues.

Conclusions: These results suggest that munitions compounds and breakdown products can affect PPARα, PPARβ/δ, and PPARγ expression in vitro and in vivo. Further studies are needed to determine the mechanisms by which these compounds affect PPAR expression.

WE 337

Molecular and behavioral endpoints in zebrafish early life stage tests: increased sensitivity and link to chemical effects

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Fish early life stage tests are based mainly on morphological changes and mortality, endpoints that might lack specificity and sensitivity to evaluate modes of action and environmental relevant concentrations. In this work we investigate if additional parameters improve the sensitivity and prediction potential of tests. Gene expression and behavior were combined to morphological endpoints to characterize toxic effects of PCB126, model dioxin-like compound. Endpoints were evaluated in two tests: (a) ELS, early life stage test using day post fertilization (dpf); and (b) pELS, prolonged ELS test. In both, fish were exposed from 3h until 5dpf to 3, 30, 100, and 300 ng/L PCB126. In the ELS, by end of exposure swimming was severely reduced and fish were recorded on video, then fish were euthanized and preserved in formalin for gene expression. In the pELS, after exposure fish were transferred to clean water, raised according to standard procedures, and observed until 14dpf, when behavior record, euthanasia and preservation were performed. Behavioral endpoints were based on previous publications. Behavioral endpoints consisted of behavior observation and activity analysis (EthoVision), which was performed on whole fish RNA with qRT-PCR using sybG, Tg-IκBα, Tg-GAPDH as housekeeping genes. Toxicity was measured by behavior score and significant differences were observed in fish exposed to 300ng/L PCB126. Gene expressions were also measured in behavioral and morphological endpoints exposed to concentrations 0.1 and 30 ng/L PCB126, and significant differences were observed in 30 ng/L PCB126 in gene expression for p38MAPK and PPARγ.

More recently, we started to combine the results of behavioral and morphological endpoints to characterize toxic effects of higher concentrations of PCB126. In this work we combined data from four ELS tests with previous data, to investigate if low and high dose-effects can be distinguished in behavioral and morphological endpoints, and to which extent behavioral and morphological endpoints are sensitive to PCB126 exposure. In the ELS, a significant decrease in swimming activity and increased mortality were observed in fish exposed to 300 ng/L PCB126. In the pELS, swimming activity and mortality were also significantly decreased in fish exposed to 300 ng/L PCB126. Additionally, a significant decrease in swimming activity was observed in fish exposed to 30 ng/L PCB126. In both tests, significant increases in expression of p38MAPK and PPARγ were observed in fish exposed to 300 ng/L PCB126. In the pELS, significant increases in expression of p38MAPK and PPARγ were also observed in fish exposed to 30 ng/L PCB126. These results suggest that behavioral and morphological endpoints can be used as biomarkers for ecotoxicological studies.

Conclusions: These results suggest that behavioral and morphological endpoints can be used as biomarkers for ecotoxicological studies. Further studies are needed to determine the mechanisms by which PCB126 affects behavior and morphology in zebrafish.

WE 338

Mechanistic profiling of flame retardants in general systemic stress and endocrine disruption within the INFLAME project

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Indoor Contamination with Flame Retardant Chemicals: Causes and Impacts (INFLAME), is a Marie Curie Initial Training Network research project funded by the EU. Its goal is to study the leaching of flame retardants (FRs) from everyday consumer goods and construction materials and their ability to enter and further influence the human body. The research of INFLAME is not solely limited to a closely related group of FRs but targets a broad range of compounds, such as brominated, chlorinated and organophosphorus FRs, as well as used chelating FRs, as they are newly emerging compounds. In this presentation, we outline the concept of an INFLAME subproject dedicated to the assessment of the FRs’ potential to cause general systemic stress and to act as endocrine disruptors. Mechanistic toxicity profiles of pure compounds, as well as indoor dust samples, are evaluated with multiple in vitro assays. A dedicated set of bacterial and human reporter gene assays are used to examine the general stress potential. These assays indicate a variety of different stresses such as specific DNA, protein- and membrane damage, as well as oxidative and oxidative stress. In order to assess the endocrine disrupting potential of FRs, we selected the MCF-7 E-screen and the H295R
steroidogenesis assay. To further understand the molecular mode of action leading to the observed effects, orbitrap-based proteomics data is gathered and combines with transcriptomics and metabolomics data acquired by other INFLAME partners. In this way, a systems biology approach is achieved. The selection of the pure compounds and sample extracts to be studied is based on chemical analysis of indoor dust samples and consumer goods. Possible mixture effects can be determined and samples, whereby biomarkers of individual bioactive FRs can not be explained by the amount of bioactive FRs present, can be subjected to further analysis. We will attempt to isolate and identify any unknown bioactive substance from these samples by combining LC fractionation and mass spectrometry with the bioassays. With this approach, more information on the specific mode of action of FRs can be obtained, that could serve as the base for future risk assessments.

**ET15P - Pesticide fate and ecotoxicology**

**WE 341**

Effects of the organophosphates, azinphos and chlorpyrifos, on juvenile fish (oryochromis mossambicus)

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Pesticides sprayed in orchards and vineyards often cause pollution of surrounding areas due to run off and spray drift and can thus reach freshwater bodies in the vicinity. The concentrations of organophosphates in the water vary with the intensity and frequency of the spraying procedures being followed. The aim of the present study was to determine whether juvenile fish occurring in such water bodies were negatively affected. Juveniles were used as little is known of their susceptibility to organophosphate pesticides and it was presumed that juveniles would be more susceptible to damaging effects of toxicants than adults. Specimens of the fish species, Oreochromis mossambicus, endemic to the waters of the study area in the Western Cape, South Africa were exposed in a laboratory experiment to field relevant concentrations of Azinphos and Chlorpyrifos. Acute tests were done and specimens were, on the other hand, exposed to an environmental exposure regime, corresponding with the programs followed by deciduous fruit farmers in the area. These programs consist of bi-weekly periods between consecutive spraying events, which are repeated up to four times during the season. LC50 values were determined and compared to values from the literature for adult fish. Exposure concentrations of 5 and 25% of the LC50 were calculated and used as exposure concentrations. The endpoints used were the whole body characteristics, mortality and growth (length and weight gain), and the biomarker, acetylcholinesterase activity. In addition, feeding response time and food consumption were measured. The results showed that Azinphos-methyl was more hazardous to the fish than Chlorpyrifos if applied on a bi-weekly basis. For the majority of endpoints investigated exposure interval played a more important role in inducing effects than exposure concentration. With shorter exposure intervals the majority of endpoints showed no difference between a higher and lower exposure concentration, while with longer exposure intervals the effect of exposure concentration became more prominent. The results of the LC50 tests indicated that juvenile O. mossambicus are more sensitive to the pesticides than adults and that different pesticide application regimes have different effects. This information could be used in the compilation of spraying programs that are used in the agricultural industry to sustain fish populations in surface waters.

**WE 342**

Carbaryl and dimethoate induced alterations in the antioxidant defense system of the aquatic snail Helisoma duryi.

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We studied the effects of carbaryl and dimethoate, individually or as a mixture, on the antioxidant defense system of H. duryi. Adult snails were exposed to 25 ppb of carbaryl or/dimethoate for 72 hours. Post mictochondial supernatants were used to determine the activity of antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), DT-Diaphorase(DTD), glutathione peroxidase (GPX) and glutathione S-transferase (GST). Thiobarbituric acid reactive substances (TBARS) levels were measured in tissue samples. The TBARS and SOD, CAT, GPX and GST activity were elevated (up to 100%) after exposure to insecticides as well as the mixture. The effects were observed after exposure to individual pesticides but were more pronounced after exposure to the mixture. Thus, carbaryl and dimethoate exposure induces an ‘oxidative stress’ in H. duryi. Altered antioxidant enzyme activity supports the use of H. duryi for the development of a potential biomarker for detecting the presence of carbaryl and dimethoate in contaminated water samples. The observed ‘oxidative stress’ suggest a potentially harmful environmental impact of these pesticides on aquatic macroinvertebrates.

**WE 343**

Does measuring cell number inhibition improve an existing high throughput algal bioassay?

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This study examines the potential use of insect behavior for detection of contaminants in water samples. Females of non-biting midge Chironomus riparius make choice of oviposition habitat for their offspring when laying eggs after fertilization in a swarm. The ability of fertilized females to detect samples contaminated with sublethal levels of chromium and chlorpyrifos in water samples was assessed in two experiments. In the first experiment, fertilized females were given a choice between two experimental chambers containing water samples that were exposed to diuron alone or a mixture of diuron and chlorazate, followed by growth and photosynthesis. What does that mean concerning the sensitivity of the 24h-biotest for herbicidal effects of realistic water samples where photosynthesis inhibiting substances as well as substances acting on cell division are likely to occur together? To clarify this question in a second step mixtures of diuron and metazachlor in different mixture ratios were analyzed. In a final cost-benefit analysis the increase of sensitivity due to the extension of the 24-h test to a 48h test including the endpoint cell division is compared to the increased costs with respect to longer test duration and higher amount of working hours needed.

**WE 344**

Oviposition preference of Chironomus riparius females - potential sublethal behavioral endpoint for in-vivo water toxicity testing

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Herbicides applied in agriculture or weed control are regularly detected in samples of surface waters. Bioassays with algae can be used to assess the effects of these herbicides to non-target aquatic organisms. For routine monitoring, such bioassays should be able to screen large amounts of low volume samples and give reproducible and accurate results within a short time period.

For this purpose Escher and Rutishauser (2008) developed a 24h-biotest using 96-well-plates for simultaneously assessing the photosynthesis inhibition and effects on growth in green algae that can be measured as optical density (OD). For herbicides inhibiting the cell division of algae, such as chloroacetanilide herbicides, this test might have a blind spot since OD mainly detects effects on cell volume growth and is less sensitive for effects on cell division. To analyze the quantitative importance of this blind spot the above mentioned biotest was expanded to the additional endpoint cell number after 48h. Two substances were selected as model compounds for this study: the phenylurea herbicide diuron, which is a known photosynthesis inhibitor and is already used as a model substance in the 24h-biotest, and the chloroacetanilide herbicide metazachlor.

Comparing the EC50 values of the different endpoints photosynthesis-, growth- and cell number inhibition identifies cell number to be the most sensitive endpoint for metazachlor, followed by growth and photosynthesis. What does that mean concerning the sensitivity of the 24h-biotest for herbicidal effects of realistic water samples where photosynthesis inhibiting substances as well as substances acting on cell division are likely to occur together? To clarify this question in a second step mixtures of diuron and metazachlor in different mixture ratios were analyzed. In a final cost-benefit analysis the increase of sensitivity due to the extension of the 24-h test to a 48h test including the endpoint cell division is compared to the increased costs with respect to longer test duration and higher amount of working hours needed.

**WE 345**

Use of Chlorella vulgaris (Chlorophyceae) to test wood preservatives at a laboratory scale

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This study examines the potential use of insect behavior for detection of contaminants in water samples. Females of non-biting midge Chironomus riparius make choice of oviposition habitat for their offspring when laying eggs after fertilization in a swarm. The ability of fertilized females to detect samples contaminated with sublethal levels of chromium and chlorpyrifos in water samples was assessed in two experiments. In the first experiment, females were given a choice between control and samples contaminated with hexavalent chromium (0.1-100 mg/L) or organophosphate insecticide chlorpyrifos (1-100 ng/L). In the second experiment (no choice experiments), fertilized females were provided with either clear control water or contaminated water sample only. In the second experiment, differences in the reproduction output of females and the reproduction output of females exposed to individual pesticides as well as the mixture. The effects were observed after exposure to individual pesticides but were more pronounced after exposure to the mixture. Thus, carbaryl and dimethoate exposure induces an ‘oxidative stress’ in H. duryi. Altered antioxidant enzyme activity supports the use of H. duryi for the development of a potential biomarker for detecting the presence of carbaryl and dimethoate in contaminated water samples. The observed ‘oxidative stress’ suggest a potentially harmful environmental impact of these pesticides on aquatic macroinvertebrates.

**WE 346**

DNA-based toxicity assay for pesticides in the environment

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Pesticides sprayed in orchards and vineyards often cause pollution of surrounding areas due to run off and spray drift and can thus reach freshwater bodies in the vicinity. The concentrations of organophosphates in the water vary with the intensity and frequency of the spraying procedures being followed. The aim of the present study was to determine whether juvenile fish occurring in such water bodies were negatively affected. Juveniles were used as little is known of their susceptibility to organophosphate pesticides and it was presumed that juveniles would be more susceptible to damaging effects of toxicants than adults. Specimens of the fish species, Oreochromis mossambicus, endemic to the waters of the study area in the Western Cape, South Africa were exposed in a laboratory experiment to field relevant concentrations of Azinphos and Chlorpyrifos. Acute tests were done and specimens were, on the other hand, exposed to an environmental exposure regime, corresponding with the programs followed by deciduous fruit farmers in the area. These programs consist of bi-weekly periods between consecutive spraying events, which are repeated up to four times during the season. LC50 values were determined and compared to values from the literature for adult fish. Exposure concentrations of 5 and 25% of the LC50 were calculated and used as exposure concentrations. The endpoints used were the whole body characteristics, mortality and growth (length and weight gain), and the biomarker, acetylcholinesterase activity. In addition, feeding response time and food consumption were measured. The results showed that Azinphos-methyl was more hazardous to the fish than Chlorpyrifos if applied on a bi-weekly basis. For the majority of endpoints investigated exposure interval played a more important role in inducing effects than exposure concentration. With shorter exposure intervals the majority of endpoints showed no difference between a higher and lower exposure concentration, while with longer exposure intervals the effect of exposure concentration became more prominent. The results of the LC50 tests indicated that juvenile O. mossambicus are more sensitive to the pesticides than adults and that different pesticide application regimes have different effects. This information could be used in the compilation of spraying programs that are used in the agricultural industry to sustain fish populations in surface waters.

The effects of the LC50 tests indicated that juvenile O. mossambicus are more sensitive to the pesticides than adults and that different pesticide application regimes have different effects. This information could be used in the compilation of spraying programs that are used in the agricultural industry to sustain fish populations in surface waters.
Environmental pollution and poisoning owing to the widespread use of pesticides in agricultural and domestic pest control may be detrimental to the health of handlers, non-target organisms and consumers. For example, some farmers preserve harvested grains by treating them with some chemicals. Pesticides or their residues are ubiquitous contaminants in the environmental media (air, soil, water), and in humans, plants and animal tissue samples. Pesticides uptake occurs through the skin, eyes, by inhalation, or by ingestion directly or through the food chain. Here we assess the genotoxic effect of dichlorvos, a widely used pesticide in Nigeria, on the DNA of poultry birds (Gallus domesticus). This study explored different special representation of treated groups using a three-path matrix model incorporating dichlorvos contamination. Thermal denaturation of the DNA from the exposed birds resulted in a significant reduction (p < 0.01) in the DNA melting temperature from 87.2°C to 81.7°C while the G/C: A/T ratio was significantly lower (p < 0.01) and the Electrophoretic (d0, d1, d2 and d3) rate in the control to 0.42 in exposed birds. Chlorophyll a/biosynthesis, in band intensity between the control DNA sample and DNA from exposed birds. These variations in band intensity were more pronounced in the RAPD-PCR products amplified with OPE-01 primer, where there is complete disappearance of DNA bands in the birds exposed to 0.04% pesticide. Thus deletion of DNA segments of birds exposed to dichlorvos can be modelled as a molecular biomarker of genotoxicity. This may also suggest that insects populations are capable of inducing genotoxic effects on non-target populations with consistent, long-term use.

WE 347
Use of passive samplers extracts for toxicity assessment of environmental realistic mixture of pesticides on natural biofilms communities
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EPOC, Arcachon, France
The objective of this study was to evaluate the potential use of passive samplers extracts in order to highlight effects of representative pesticide mixtures on natural biofilm communities.

Chronically high levels of common household pesticides are present in the environment, often in the range of micrograms per liter, concentrations known to cause toxic effects on aquatic invertebrates, particularly aquatic biofilms. Chronically high levels of common household pesticides are present in the environment, often in the range of micrograms per liter, concentrations known to cause toxic effects on aquatic invertebrates, particularly aquatic biofilms. The impacts of pesticides and their metabolites on aquatic biofilms have been extensively studied, particularly on their structural attributes (e.g., density and diversity of diatoms). However, the effects of pesticides on ecological attributes (e.g., structural diversity and functional activity) of aquatic biofilms have received less attention. The objective of this study was to evaluate the potential use of passive samplers extracts in order to highlight effects of representative pesticide mixtures on natural biofilm communities.

Our results underline impacts of pesticides mixture on both growth (DW, AFDM and diatoms density) and community tolerance of a natural biofilm. In the one hand, however, in many cases reapplication is required to provide effective season-long control. A new slow-release, wax-based formulation of novaluron has been developed by Pestalto Inc. to regulate the release of the active ingredient in standing water allowing for a prolonged mosquito control period. This novaluron formulation was added to a Biofilm to test the effects of different concentrations. The concentrations of released novaluron measured by biofilm, however the following pattern was recognized: endosulfan made cladocerans more capturables (p < 0.05) but copepods enhanced their escape ability at lower concentrations (p > 0.05). The mayfly Cloeon dipterum is a very promising aquatic insect for use in chronic exposures.

WE 348
A chronic exposure test method for two aquatic insects, the mayfly Cloeon dipteron and the caddis fly Anabola nervosa
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Species sensitivity distribution (SSD) models are an important tool in protecting aquatic ecosystems. Most SSD models rely on acute toxicity endpoints. Some of the reasons for this include: (i) testing requirements are clearly defined and easy to follow; (ii) chronic endpoints may be more sensitive and accurate in predicting chronic impacts, however, in many cases reapplication is required to provide effective season-long control. A new slow-release, wax-based formulation of novaluron has been developed by Pestalto Inc. to regulate the release of the active ingredient in standing water allowing for a prolonged mosquito control period. This novaluron formulation was added to a Biofilm to test the effects of different concentrations. The concentrations of released novaluron measured by biofilm, however the following pattern was recognized: endosulfan made cladocerans more capturables (p < 0.05) but copepods enhanced their escape ability at lower concentrations (p > 0.05). The mayfly Cloeon dipterum is a very promising aquatic insect for use in chronic exposures.

WE 349
Behavioural toxicity of endosulfan and a novel approach to the study of its ecotoxicity in aquatic microcrustaceans
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Despite the organochlorine insecticide endosulfan is currently banned in over 30 countries, it is still widely used in most Latin American countries. Recently, values from 0.2 to 10.9 µg/L have been measured in neotropical streams, exceeding the acute toxicity threshold. Its continued use might represent the hazardous agonistic contexts, despite several works have demonstrated its negative consequences to humans and environment. In most cases, it is directed to non-target organisms it has also been reported indirect effects, such as alterations in predator prey interactions, in aquatic and terrestrial organisms. The importance of these last studies lies in providing relevant information on the actual ecological processes that take place after agronomic interventions. The purpose of this work was to demonstrate an indirect effect of the early impact of an endosulfan based-insecticide on the escape behaviour of four subtropical microcrustaceans: two copepods (Notodiaptomus conifer, Argyrodiaptomus calci) and two cladocerans (Pseudodiaptomus variabilis and Ceriodaphnia dubia). The experimental assays were carried out using a novel hydraulic devise designed to mimic the fungal pathogen Phoma betae. The biological effects were relevant. Capture biofilm. This allows to quantify the organisms’ ability to perceive and react to the approach of possible predators. Two environmental relevant concentrations of endosulfan were used. They were prepared from a commercial formulation containing 35% of the active ingredient (Zebrak Cream®). Endosulfan concentrations in water was quantified by GC-ECD showing recoveries >95% of the nominal value. The exposure time was 15 (5) minutes. Analyses of data were carried out using the Deviance as variability means (ANODEV). The magnitude and the kind of responses were different depending on species, capture speed and toxic concentration, however the following pattern was recognized: endosulfan made cladocerans more capturables (p < 0.05) but copepods enhanced their escape ability at lower concentrations (p < 0.05), which might be the result of an early toxicant stimulation. The immediate apparent advantage of this last effect will be discussed in the context of ecophysiological theories, as it can be an important means by which the fish population is reduced. Considering the comparatively high sensitivity of the escape, the proposed method is suggested as an effective complement (and ecologically relevant) in ecotoxicology.

WE 350
Characterization of a controlled release formulation of Novaluron
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Mosquitoes pose a serious risk to public health as vectors of human diseases such as the West Nile fever, malaria and dengue fever. In 2009 there were 225 million cases of malaria worldwide; these cases resulted in the death of 781,000 people. Consequently, it is extremely important to control mosquito populations especially at their larval stage. L-0 mosquitos to measure its efficacy against target (mosquito larvae) and non-target (zoooplankton) species. Mesocosms were divided into 5 groups: novaluron (0.12% 300g/mesocosm) with added sediment, novaluron (0.12% 300g/mesocosm) without added sediment, novaluron (0.48%)300g/mesocosm) with added sediment, novaluron (0.48%)300g/mesocosm) without added sediment and no novaluron with added sediment. The concentrations of released novaluron measured in each mesocosm by Biofilm were tested for statistical differences using a one-way ANOVA. After 28 days, however, compared to the concentration of 50 µg/L, dichlobenil treatment provided 100% control of Aedes andophytes throughout the 120 day study while the 0.12% treatment provided 100% control for only 28 days after application. Other monitored parameters were affected by the addition to novaluron to the mesocosms; dissolved oxygen and chlorophyll concentrations were higher in sampled water containing novaluron, while weight, the flaked fish food suspension along with the green algae was chosen as an appropriate diet for both species. The final study design was with a 28-day study duration, 5 replicates/treatment and 8 individual/time replicate, under static renewal conditions (renewals on Monday, Wednesday and Friday) in laboratory dilution water and laboratory dilution water with co-solvent. A new slow-release, wax-based formulation of novaluron has been developed by Pestalto Inc. to regulate the release of the active ingredient in standing water allowing for a prolonged mosquito control period. This novaluron formulation was added to a Biofilm to test the effects of different concentrations. The concentrations of released novaluron measured by biofilm, however the following pattern was recognized: endosulfan made cladocerans more capturables (p < 0.05) but copepods enhanced their escape ability at lower concentrations (p < 0.05), which might be the result of an early toxicant stimulation. The immediate apparent advantage of this last effect will be discussed in the context of ecophysiological theories, as it can be an important means by which the fish population is reduced. Considering the comparatively high sensitivity of the escape, the proposed method is suggested as an effective complement (and ecologically relevant) in ecotoxicology.

WE 351
Effects of dichlobenil on mRNA transcription of thyroid hormone regulation in Chinese rare minnow (Gobiocypris rarus)
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Dichlobenil (3,6-dichloro-2-methoxybenzoicacid) is a benzoic acid herbicide and widely used in agriculture, which has been detected in surface-water and groundwater. In this study, to determine the endocrine disruption effects of dichlobenil, adult fish were separately exposed to concentrations 0.03, 0.05, 0.5 and 50 g/L. The results showed that no effects on length in adult males, whereas the length of adult females were significantly decrease in 50 µg/L dichlobenil treatment (p<0.5). Moreover, GSI and
Acute exposure of a glyphosate-based herbicide affects the gills and liver of the Neotropical fish, Piaractus mesopotamicus

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The glyphosate (N-phosphonomethyl glycine) herbicides, post-emergent herbicides with a broad-spectrum and low selectivity, have been intensively applied on numerous field cultures, urban and industrial areas to control unwanted plants, including the macrophytes in aquatic systems. Recently, a new glyphosate formulation Roundup® Ready (RR) was specifically developed for genetically modified glyphosate-tolerant soybean crops (RR crops), was commercially available in Brazil. This study determined the acute toxicity of such herbicide in Piaractus mesopotamicus, and evaluated the effects on the morphology of the gills and liver of exposed surviving fish. Fish were exposed to 3.0, 3.5, 4.0 and 4.3 mg L-1 of glyphosate, and the LC50(48h) was estimated at 3.74 ± 0.2 mg L-1. Gill histopathology was rare, and the activity of Na+K+ ATPase enzyme did not change, function was classified as moderate to severe. Cytoplastic vacuolization, lipid accumulation, nuclear membrane alterations and glycogen depletion were found in the liver, suggesting a reduction in the liver function. The glyphosate in the RR formulation was classified as moderately toxic for P. mesopotamicus, and the severe damage in the liver may affect the detoxification and/or tissue repair process and contribute to fish death.

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WE 354

Effects of the fungicide tebuconazole on fungal and bacterial communities in the sediment of outdoor freshwater microcosms

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Only a few studies have been published on the non-target effects of fungicides on aquatic microbial communities. One reason for the lack of information regarding effects of fungicides on microbial communities may be the basic dossier requirements for EU registration. For zooplankton, invertebrates and primary producers are asked for the aquatic effect assessment. Aquatic fungi and bacteria, however, play an important role in freshwater ecosystems, contributing significantly to decomposition processes. Hence, aims of the present work were: (1) to evaluate the impact of tebuconazole on sediment microbial community structure of an experimental freshwater ecosystem, and (2) to evaluate whether the current effect assessment procedure (based on standard tests with fish, aquatic invertebrates and algae) is protective for aquatic fungi and bacteria. To this end, outdoor microcosms were set up in an experimental ditch and four test systems served as controls while tebuconazole was applied to four other microcosms at an intended concentration of 238 µg a.i./L. This treatment level is in line with the HC5 (based on acute toxicity tests with aquatic microorganisms) for tebuconazole, as described by Malby et al. (2009). Throughout the experiment sediment samples were taken from each microcosm and used for total DNA isolation. The isolated DNA was used to access bacterial and fungal community structure by Denaturing Gradient Gel Electrophoresis (DGGE) and 454 pyrosequencing of PCR-amplified ribosomal RNA gene fragments. Results showed that fungal community structure was not affected by the treatment, whereas controls and treated systems presented a similar 21d exposure test formula. DGGE profiles of controls supposed potential endocrine disrupter, for example methymeth and endosulfan.

WE 355

Chronic toxicity of acetone, DMF and DMSO used in sediment-water test systems on Lumbriculus variegatus

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Organic solvents are frequently used for test items with low or no water solubility. According to the OECD Guideline 225 (Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment) a suitable organic solvent for the application of test items should be used. However, until now no data on the toxicity of solvents listed in OECD series No. 23 on Lumbriculus variegatus are available. Toxicity data for the following organic solvents will be presented: Dimethylformamide (DMF), Dimethylsulfoxide (DMSO), Acetone.

WE 356

Oxidative stress, acetylcholinesterase and physiological status as responses in clams transplanted into the Mar Menor lagoon (Spain) to assess the biological impact of anthropogenic activities

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The Mar Menor Lagoon (SE Spain) is subjected to several forms of disturbance, amongst which chemical pollution associated with anthropogenic activities (industrial production, urbanization, tourism, recreation). Previous works showed the input high levels of zinc and organophosphate pesticides to this lagoon through the El Albuñol watercourse from different sources (groundwaters, wastewater effluent, etc.). Therefore an environmental monitoring is necessary to protect biological/ ecological systems, and study the overall biological effects of exposure to potentially harmful substances. In the present work, an active biomonitoring approach (ABM) was performed using clams Ruditapes decussatus collected at a pristine site, and transplanted to four locations inside the lagoon, two of them near to the Albuñol wadi mouth, for 7 and 30 days in autumn 2009. The primary aim of this study was to select a set of relevant biomarkers in transplanted clams for the biological assessment of water pollution in the Mar Menor. Oxidative stress, neurotoxicity and physiological responses were studied in these biomarker organisms: levels of catalase (CAT), glutathione reductase (GR), glutathione S-transferase (GST) and lipid peroxidation (LPO) were measured in digestive gland tissues, acetylcholinesterase activity in gills of clams before and after the transplant experiment. The physiological status of the animals was assessed by the scope for growth (SFG). Clams exposed to high levels of Albuñol appeared to be the most impacted by the pollutant discharges. Biochemical responses displayed a high oxidative stress, with significantly higher levels of LPO, GST and GR than those found at time 0 and other sampling sites, whereas ACHE activity was significantly inhibited by the input of organophosphate pesticides. Physiological rates were also affected at the sites near the pollutant discharges where a significant decrease of 10% on the SFG were recorded. Thus these results suggested that ABM approach have a potential for application in the Mar Menor pollution monitoring.

WE 357

The effects on non-target species of novel products applied together with herbicides and insecticides in relation to transgenic soybean crops in Argentina

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Aquatic environments of the pampasic region of Argentina receive chemicals from agricultural activity due to an increase in a glyphosate tolerant transgenic variety of soybean crops. In the last year the extension of the soybean crops in the region has reach to about fourteen million hectares. Non-target organisms, as aquatic primary producers are severely affected by this agricultural contamination. The cultivation of transgenic soybean is an activity of real importance to the national economy, thus the use of chemicals in order to reduce those that occur as plant and animals pests has increased. The research for new products for this purpose is under constant development.
The present study is aimed to compare the toxicity of herbicide Glyphosate and insecticide Endosulfan and Chlorpyrifos prior and after the addition of three novel products which formulations is based on nonylphenol ethoxylates as adjuvant. These products are mixed with pesticides according to rates indicated in the product containers, whose function is to enhance penetration, avoid drift and evaporation improving the biological activity of pesticides. The not target organisms selected were the aquatic primary consumers Daphnia magna and the floating macrophyte Lemna minor, as they are essential to ecosystem providing the basic energy for food webs. The comparisons were made under a range of concentrations at which formulations is based on nonylphenol results in more severe and serious effect of major significance at the population and ecosystem level.

**Evidence of toxicities of herbicides using short-term chronic tests of alga, daphnids and fish**

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To calculate the possible adverse effect of pesticides on our ecosystem, the concentrations and application window of herbicides in the environment were needed to be monitored. The aim of this study was to compare the toxicity of herbicides in environment were characterized by chemical analysis. The agricultural drainage water collected from 14 different spots of rice fields in Japan. Water samples were collected directly after 7 days of herbicide application. The short term toxicity chronic tests were carried out using algae (P. subcapitata) growth inhibition test, daphnid (C. dubia) reproduction test, and fish (D. rerio) short-term toxicity test on embryos and larvae stages. In this study, the effects on algae and daphnids were observed in 9 spots and 2 spots, respectively. The effect on hatching, survival of fish was not found in any spots examined. We also performed simultaneous analysis of the GC-MS for the water samples, and 92 different herbicides were detected in this study. Bromobutide was detected at high concentration in almost all fields. Dimethametryn and pretilachlor were also detected in the same spots. One month later from herbicides application, the concentrations of herbicides detected in this study decreased significantly. Our results suggested that the herbicides disappear immediately by deintegration or proliferation in the environment. Moreover, we examined the short-term toxicity testing of the GC-MS for 3 species for candidate herbicides, which were detected in the water samples in this study. In this study, the strongest effect was obtained in the algae. Toxicity on the algae was found in pretreatment, cyanoazide and simetryn at 1–10 mg/L. The effects of herbicides on daphnids were weak, and the treatment of herbicides, exposure time, and concentrations were not found to be significant. In daphnids, no significant toxicity was observed at 100 times of concentration compared to an alga. The effects of herbicides were found in order of alga > daphnid > fish. In the present study, it suggested that the short-term chronic toxicity tests using the aquatic organisms is effective in predicting the effect of herbicides released in environment. For the chemical safety and health management of environmental chemicals including herbicides, further study is needed to detect synergistic effects with other herbicides and pesticides.

**Mutagenic and genotoxic effect of Roundup Transorb® herbicide in gill cells and erythrocytes from Pocella reticulata**

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The effects of mutagenic and genotoxic substances in the fish genome have been the subject of many studies, especially those that seek to establish the response of genes to environmental stimuli. This study aimed to conduct a study on mutagenicity and genotoxicity in fish Pocella reticulata, by exposure to Roundup Transorb® using the micronucleus and Comet assay. Erythrocytes were collected from the gills of the fish treated with the herbicide. Fifteen fish were used for each treatment at the following concentrations: 0 µl/L, 1.41 µl/L, 2.83 µl/L, 4.24 µl/L and 5.65 µl/L at 24 hours of exposure. For the analysis of slides, 75 cells were counted and averaged to the frequency of the Nuclear Morphological Changes (NMAs). In the MN test and Comet assay, the minor was subjected to significant genotoxic and mutagenic effects in the gills of gills and cells, cellular gradual increase in the number of cells with NMAs and DNA damage under higher concentrations, indicating a concentration-dependent effect. These results suggest that the formulation of the herbicide tested showed genotoxicity and mutagenicity tests for the MN and Comet assay, and they are very efficient in detecting low levels of contamination.

**Acute toxicity of the herbicides ametryn, Glyphosate and it is association (Glyphosate+Ametryn) to Daphnia magna**

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In Brazil it very common the application of herbicides in association (tank mix) to improve the range of action and to reduce the costs. This practice is not prevened in laws. This study was aimed to evaluate the behavior of associations of herbicides in aquatic organisms. The results showed that the herbicide association is a non-toxic transformation product and had been been selected, being known as "environmental friendly" with quick mineralization and low toxicity. Ametryn is an herbicide of residual effect, with moderated toxicity and environmental persistence. The association between this two herbicides could be synergic or antagonistic about its environmental mineralization, degradation, persistence and toxicity. The aim of this study was to evaluate the acute toxicity of the herbicides Ametryn, Glyphosate and Ametryn + Glyphosate to the microcrustacean D. magna. Firstly, was realized a preliminary test for each product in order to establish the concentrations to be used in the definitive acute test, which were: 10, 20, 40, 60, 80 and 100 mg/L for each product and to the association (glyphosate+ametryn). All concentrations were conducted in two replicates with five organisms each; plus control (reconstituted water). The test duration was 48 hours, in a dark incubation chamber without food. After this period the Median Effect Concentration (EC50) for each product was calculated using the Probit program Trimmed Spearman Karber (Hamilton et al., 1977). The EC50 (48h) for ametryn, glyphosate and ametryn + glyphosate were respectively (mg/L): 41.35; 28.8 and 28.28. We conclude that glyphosate and the mixture ametryn + Glyphosate were more toxic than ametryn. The mix of ametryn and glyphosate did not increase or reduce the toxicity of glyphosate and the mix of ametryn + glyphosate. A chronic toxicity test is being conducted and the results should be presented at the poster in the congress.

**Inhibition of oxidative stress enzyme activities and genotoxicity of the insecticide imidacloprid in the freshwater fish Australoheros facetus**

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Imidacloprid (IMI) is a neonicotinoid insecticide with neurotoxic action that is at the moment one of the insecticides with the world's fastest growing sales. In this study, biochemical and genetic parameters of the freshwater fish Australoheros facetus after acute exposure to IMI are presented. Glutathione-S-transferases (GST) and catalase (CAT) activities, malondialdehyde content (MDA) in liver and gills and micronucleus frequency (MN) in blood were analyzed. Fish were exposed to: 0 (negative control), 1, 10, 100, 1000 µg/L during 24 h. Biochemical parameters were measured spectrophotometrically. Peripheral blood smears, two per fish, were made by applying a drop of blood on clean slides, fixed in absolute methanol; air dried and stained with 15% Giemsa solution. Two thousand erythrocytes, 1000 per slide, were analyzed from each animal under 1000 X magnification. In gills GST and CAT activity was inhibited at 10, 100, 1000 µg/L and at 1, 10, 100, 1000 µg/L, respectively (p<0.05). In liver, stress defense enzymes at mostly all the concentrations tested as well as mortality of fishes at 10 mg/L after 3 hours of exposure. The responses observed in our work when fishes were exposed to environmental relevant concentrations, alert about the possible impact of these levels in aquatic ecosystems and highlight the different sensitivity of the fish species.

**DNA damage induced in fish (Anguilla anguilla) by aminomethylphosphonic acid (AMPA) - the major breakdown product of glyphosate**

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Most studies on the effects of pesticides in aquatic organisms are focused on the active ingredients of the commercial formulations. However, the assessment of the direct impact of chemicals that may occur in the environment as breakdown products of the parental compound could be considered even more ecotoxicologically relevant. Glyphosate is the active ingredient of various well-known herbicide preparations applied worldwide, that easily reaches the aquatic compartment where it can be naturally degraded and unable to produce synergistic effects with herbicides and pesticides. This practice is not previewed in laws. The aim of this study was to assess the DNA damage potential of AMPA in fish and compare it with that of glyphosate. Therefore, the comet assay was performed for the first time being applied to blood cells of Anguilla anguilla. After 24 hours of exposure, we observed that only lower concentration of glyphosate showed to induce DNA damage. Thus, the present findings confirmed the genotoxicity of AMPA, for the first time in fish. However, this metabolite appears to be more dangerous after 1 day, since it was possible to observe a temporal recovery along the experiment. Moreover, there are no evidences that AMPA is more genotoxic than its precursor glyphosate.
represent a potential threat to fish, their genotoxic potential remains largely unexplored in this animal group. Moreover, genotoxic studies have been almost exclusively focused on the active ingredients, whereas the affects of adjuvants are frequently disregarded.

Therefore, the present work addressed the broad-spectrum herbicide - Roundup® - as one of the most popular formulations worldwide, aiming the evaluation of the relative contributions of the active ingredients (glyphosate and amine; POEA) to the genotoxicity of the commercial mixture on Anguilla anguilla. Fish were exposed to equivalent concentrations of Roundup® (58 and 116 µg/L), glyphosate (17.9 and 35.7 µg/L) and POEA (9.3 and 18.6 µg/L), during 1 and 3 days. The comet assay was applied to blood cells. After 1 day exposure, it was observed that all treatments, with the exception of the lower concentration of Roundup®, displayed significant DNA damage in comparison with the control. Results in relation to 3 days exposure, significant increases were observed for the higher concentration of Roundup® and the lower concentration of glyphosate, as well as for both POEA exposed groups.

Overall, the results confirmed the genotoxicity of Roundup®, also demonstrating the DNA damaging potential of glyphosate and POEA individually. Though both components contribute to the overall genotoxicity of the pesticide, their individual effect was not observed, suggesting an antagonistic interaction. Though POEA is far to be considered biologically inert, did not increase the risk associated to glyphosate. The present findings emphasize the need to define regulatory thresholds for all the formulated products, required to consider, in particular, the revision of the hazard classification of POEA.

**WE 364**

Bioaccumulation and toxicity of the herbicide Prowl® in Daphnia magna exposed to contaminated food


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The intensive use of pesticides leads to the contamination of soils and nearby water systems due to runoff, leaching or spraydrift from agricultural areas. Pendimethalin - the active ingredient of Prowl® - is a herbicide used before crop emergence or planting, since it inhibits the development of weeds in seedlings. Therefore, besides attaining freshwater reservoirs, pendimethalin may also affect green algae in particular, what in turn may influence the quality of the food for the majority of herbivorous zooplankton, affecting their ecology and community structure. The aims of this work were: i) to evaluate the nutritive quality of phytoplankton previously exposed to sub-lethal concentrations of Prowl®; ii) to assess changes on the reproductive output of D. magna cultured in non-contaminated ASTM medium and fed with Pseudokirchneriella subcapitata previously exposed to sub-lethal concentrations of Prowl® and iii) to evaluate the bioaccumulation of pendimethalin by D. magna.

First an algae growth inhibition test was performed to evaluate the ecotoxicity of Prowl® for P. subcapitata. EC50 and EC20 values obtained for growth inhibition were 18.3 ± 1 g L⁻¹ and 10.7 ± 1 g L⁻¹, respectively. Thereafter, P. subcapitata was cultured in MBL medium containing a concentration of pendimethalin equivalent to the EC20 value, to obtain contaminated algae. These were used to feed D. magna during the chronic assay. After 26 days, the results expressed a significant decrease in the fecundity of daphnids that were fed with contaminated algae. However, no significant differences in the growth rate of D. magna were recorded, when compared to the control (with no contaminated food). In what concerns the bioaccumulation of pendimethalin, it was observed that this herbicide had a significant bioaccumulation potential (> 4.20 mg/g) in daphnids. The current results highlighted that low concentrations of Prowl®, that could be found under real-scenarios of agricultural areas, can affect the reproductive performance of D. magna, due to the change of their food quality.

**WE 365**

Differential gene transcription in Pococila vivipara exposed to the herbicide Atrazine


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**WE 366**

Effects of Plant Protection Products on Lemna minor growth - active ingredient vs. formulated compounds

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The intensive use of pesticides leads to the contamination of soils and nearby water systems due to runoff, leaching or spraydrift from agricultural areas. Pendimethalin - the active ingredient of Prowl® - is a herbicide used before crop emergence or planting, since it inhibits the development of weeds in seedlings. Therefore, besides attaining freshwater reservoirs, pendimethalin may also affect green algae in particular, what in turn may influence the quality of the food for the majority of herbivorous zooplankton, affecting their ecology and community structure. The aims of this work were: i) to evaluate the nutritive quality of phytoplankton previously exposed to sub-lethal concentrations of Prowl®; ii) to assess changes on the reproductive output of D. magna cultured in non-contaminated ASTM medium and fed with Pseudokirchneriella subcapitata previously exposed to sub-lethal concentrations of Prowl® and iii) to evaluate the bioaccumulation of pendimethalin by D. magna.

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**WE 367**

Assessment of the effects of the 2010-2011 Victorian locust control program on aquatic ecosystems

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In spring 2010, Victoria, Australia was threatened with a potentially serious locust plague. In response, the Victorian Government launched the Australian Plague Locust Response (APLR) to mitigate locust impacts by biological and chemical control. The aim of this study was to assess and compare the effects of the active ingredient and respective formulated product of an herbicide (pendimethalin vs. Prowl®) and a fungicide (copper sulphate vs. Calda Bordalesa®) on the growth of Lemna minor. The ecotoxicological evaluation of formulated products gives a hint of potential risks under real agricultural scenarios. The biomass production of L. minor used for the calculation of growth rate was based on dry weight measures.

Overall, dose-response relationships were determined for all the compounds tested. In general, the formulated products were more toxic, to L. minor growth than the respective active ingredients. In particular, Prowl® induced quite significant growth rate impairments on L. minor, since it inhibits microtubule formation during cell division. In spite of this, the active ingredient of the fungicide was more toxic than that of the herbicide, evidencing a lower EC50 value. Anyway, the generally reduced effect concentrations were determined to be < 4 mg L⁻¹ for all formulated products. Future studies should extend the testing for other sub-lethal responses and macrophyte species, which usually have important roles in ecosystem balance namely related with the natural mitigation of agrochemicals entering the aquatic systems.

**WE 368**

Ecotoxicity of triclosan, triclocarban and other antimicrobial agents to aquatic organisms and benthic invertebrate

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Antimicrobial agents such as 2-pheoxyethanol and p-thymol. Additionally, triclosan and triclocarban was relatively hydrophobic and accumulative in sediment, and several researchers have detected them from sediment.

In this study, we investigated acute and sub-chronic toxicity of six compounds (Triclosan, triclocarban, p-Chloro-m-xylene, 4-isopropyl-3-methylphenol, 2-
phenoxyethanol and resorcinol) using conventional three aquatic organisms, fish, daphnids, algae and a benthic invertebrate chironomid in addition to the measurements of these compounds in urban streams in Tokushima, Kyoto, and Saitama, Japan. Combining these ecotoxicological and monitoring data, we conducted screening level ecological risk assessment of these compounds.

Triclosan was found to be very strongly toxic for algae with 96 h-EC50 of 2.8 µg/L but moderately toxic for daphnids and fish. Triclocarban showed the similar trend but was more strongly toxic to daphnids and fish than triclosan. Other antimicrobial agents were less toxic than these two with a few exception such as resorcinol, which was relatively toxic to daphnids (8-DOE of 310 µL/L). As presented above, the toxicity of triclosan and triclocarban was found to be two to three orders of magnitude lower than the toxicity of phenoxethanol and resorcinol on the same aquatic organism. The obtained results showed that microbial compounds may have additive effects as we observed for parabens, another class of preservatives.

Further, investigating the antimicrobial agents necessary by the information on the rate and ecological risk in the urban area is still limited. In addition, these compounds may have additive effects as we observed for parabens, another class of preservatives.

WE 369

Acute ecotoxicity evaluation of plant extracts such as pepper extract, zanthoxyln extract, clove bud oil, rosemary oil, and organum oil


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Environment-friendly agro-materials tend to be used for pest control. For this reason, many studies are conducted to develop environment-friendly insecticides containing natural materials. The purpose of this study was to assess ecotoxicity for 5 plant extract and essential oils (pepper, Zanthoxylum, clove bud oil, rosemary oil, and organum oil) expected to use as antimicrobial agents in the microcosms. The obtained results were used for the test on different species.

Target species were chopped into sections 1:1, were sprayed with herbicide sprays solutions equivalent to 0.001-0.1 of field application rate. The aquatic organisms were affected by the same concentrations of herbicide in their growth medium separately and in the microcosms (L. minor + P. subcapitata; L. minor + P. subcapitata + D. magna).

Based on this concern, this communication proposed the use of chemical transformation of flavonoid isolated from citrus pulp to control ants without affect other insects.

The flavonoid underwent a conversion of its carbonyl function to an imine. The obtained compound presented high inhibition of L. gongylophorus growth. This compound material will transform in food to the nest and it will disappear.

WE 370

Effects of the sulfonilurea herbicide amidosulfuron on target and non-target terrestrial and aquatic organisms

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The usage of herbicides is increasing in agriculture and herbicides have significant environmental and human health impact. Risk assessment of chemicals is often based on single species tests under optimal laboratory conditions for tested organisms. One of the major drawbacks of these tests is that they do not account for species biological interaction and natural conditions which are often non-optimal. The effects of the sulfonilurea herbicide amidosulfuron on target and non-target organisms: plants (Chenopodium album, Hordeum vulgare; Lemma minor), algae (Pseudokirchneriella subcapita and Daphnia magna) were tested using single- and multi-species systems. The terrestrial target Ch. album and non-target herbivore plants, growing separately and together in the microcosms at the concentration 1:1, were sprayed with herbicide at several doses up to 0.001-0.1 of field application rate. The aquatic organisms were affected by the same concentrations of herbicide in their growth medium separately and in the microcosms.

The obtained results were used for the test on different species.

Some differences between the response of individually grown terrestrial plant species and the same species grown in the microcosms were observed. In two-species tests the impact of herbicide on growth and photosystems of Ch. album was less pronounced than in single-species tests, as in the microcosms the herbicide at the same time was present in the different body parts (head, thorax, abdomen) but were preferentially concentrated in the abdomen.

The concept of bioremediation pest control, a way to control insects with the use of natural products and their derivatives, is proposed in order to minimize the environmental impacts. The symbiosis between leaf-feeding ants and the fungus Leucogastromycetes gongylodes has been used to control the ants, since if the fungus die plants material will transformed in food to the nest and it will be disappear.

Based on this concern, this communication proposed the use of chemical transformation of flavonoid isolated from citrus pulp to control ants without affect other insects.

The flavonoid underwent a conversion of its carbonyl function to an imine. The obtained compound presented high inhibition of L. gongylophorus growth. This compound material will transform in food to the nest and it will disappear.
The effect of insecticide resistance to environment in grape production
S. Merrem, M.U. Usuly, C.T. Turgut
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European grapevine moth (EGM) is the most common pest in the vineyards of all over the European and Mediterranean countries. The moth reduces yields and quality of vineyards. Insecticides are used season to control the moth in vineyards up to 5 times from mid-season to end of the and effect nontarget organism and pose risk in the environment. The most important danger is insecticide resistance of EGM because of higher application rates and use frequency of insecticides. The aim of this study was to explore the effect of the risk factors, bees etc., by using Environmental Impact Quotient (EQ). The larvae were collected from 3 different places; Aladkebir, Salihli, Sarıgöl, where the intensive viticulture farming of the Aegean region. All population were maintained in a climate chamber. EC50 values of insecticides were measured by using enymatic and bioassay methods. Due to the occurrence of resistance, how often pesticide applications are required and the effect of this situation on non target organisms will be estimated by using EQ:

We 375
Trubltinum (TBT) bioremediation evaluation in soil after Aeromonas molluscumca Av27 inoculation
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Due to its wide application on antifouling paints and industrial agriculture processes Tribultinum (TBT) has become the most significant pesticide in marine ecosystems. Despite the global ban of its use in paints by 2008 in the International Maritime Organization, TBT remains a worldwide pollution problem due to its high persistence, toxicity and bioaccumulation owing to its high affinity to suspended particles and sediments. Several studies have shown that TBT contamination and their disposal constitutes an important pathway for this compound to reach soils and affect soil fauna and flora. Considering this, biological degradation seems to be one major tool to remove TBT from the environment. Aeromonas molluscumca Av27 is an bacterium that is able to degrade TBT in aqueous solutions into its less toxic byproducts, dibutyltin (MBT) and monobutyltin (MBT). Therefore, it can be used as a powerful natural tool to diminish contamination of TBT in the environment. In this study we aimed to evaluate the ability of A. molluscumca Av27 to bioremediate TBT contaminated soil. With that purpose, after remediaiton of soil by Av27, two bioassays were performed:

1: the seed germination and growth test with the rapid cycle turnip Brassica rapa, ii) the feeding inhibition test with the terrestrial isopod Porcellio spinicornis. Decrease in Above results was also evaluated using a simple method based on the inhibition of growth of an indicator Micrococcus luteus strain. Several trials were carried out using different approaches for A. molluscumca Av27 inoculation.

The following results were obtained: in the plant bioassays, in the presence of A. molluscumca Av27 was observed a decrease in the number of germinated seeds however, it stimulated plant growth within the range of TBT concentrations; the feeding bioassay revealed higher consumption ratios on isopods exposed to soil inoculated with Av27, when compared to the control exposed to soil without bacteria, within the TBT concentration range. These experiments can bring new insight for bioremediation methodologies for TBT contaminated soils.

We 376
Details on occurrence and frequency of guttation in different crops in Germany
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In crop species of economic relevance in Germany, several glasshouse and field trials were conducted from 2009 to 2011 by the Julius Kuhn-Institut in Germany and by cooperating institutions (DWD, IfZ, RUP and UFOP) to determine the frequency and occurrence of guttation. Several crop species such as sugar beet, winter oilseed rape, maize, barley, potatoes, oat, onions, carrots, peas and cucumber were investigated and an assessment of the occurrence, frequency and intensity of guttation (size/number of guttation drops, number of guttating plants) in the tested crop species was conducted. Also the formation of dew was assessed. Observations were conducted from the beginning stages of the growing season until at least the flowering stage or until at least the fruit formation stage was recorded. In the glasshouse trials, the size of guttation drops was determined by weighing of the guttation drops. In the field trials, sky cover and soil humidity were estimated. In the field, the guttation frequency of adventage vegetation was also assessed in order to facilitate a comparison to the investigated crop species. Daily assessments were conducted in the glasshouse while assessments in the field were only conducted under climatic conditions suitable for guttation.

Guttation occurred mainly at young growth stages in the tested crop species. Only maize, cucumber and potatoes produced guttation droplets up to emergence of (MBT) and monobutyltin (MBT). Therefore, it can be used as a powerful natural tool to diminish contamination of TBT in the environment. In this study we aimed to evaluate the ability of A. molluscumca Av27 to bioremediate TBT contaminated soil. With that purpose, after remediaiton of soil by Av27, two bioassays were performed:

1: the seed germination and growth test with the rapid cycle turnip Brassica rapa, ii) the feeding inhibition test with the terrestrial isopod Porcellio spinicornis. Decrease in Above results was also evaluated using a simple method based on the inhibition of growth of an indicator Micrococcus luteus strain. Several trials were carried out using different approaches for A. molluscumca Av27 inoculation.

The following results were obtained: in the plant bioassays, in the presence of A. molluscumca Av27 was observed a decrease in the number of germinated seeds however, it stimulated plant growth within the range of TBT concentrations; the feeding bioassay revealed higher consumption ratios on isopods exposed to soil inoculated with Av27, when compared to the control exposed to soil without bacteria, within the TBT concentration range. These experiments can bring new insight for bioremediation methodologies for TBT contaminated soils.

We 377
Assessment of exposure to polycyclic aromatic compounds (PAcs) in avian embryo hepatic C-oxidation system
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Polycyclic aromatic compounds (PAcs) are considered to be ubiquitous environmental pollutants. For instance, many polycyclic aromatic hydrocarbons (PAHs) are well-known to be potentially carcinogenic to humans and ecotoxicological effects have been shown in many studies. However, PAHs can also be oxidized into more soluble oxygenated derivatives (Oxy-PAHs) and these derivatives have been reported to have hormone disturbing potency, showing mutagenicity in bacterial and human cell and also inducing potentially carcinogenic Oxy-PAHs form, only primarily during incomplete combustion, but also following secondary oxidation of PAHs in the atmosphere and during chemical and biological degrading processes of PAH-contaminated soil. Although some studies have addressed questions concerning the health impact of PAHs in avian over the years, the number of studies investigating avian toxicity of Oxy-PAHs is practically non-existent. We have designed a method to investigate the effects of organic pollutants such as PAHs on the C-oxidation of fatty acids in hepatic embryonic tissue from chicken (Gallus domesticus). Our previous studies provide direct evidence that the metabolic effect on hepatic C-oxidation system is clearly reduced after exposure in ovo to a 16 PAHs mix, showing decreased oxidation values compared to control. The focus for this present study is (a) exposure to a 13 oxygenated PAHs mix, (b) single exposure to some selected PAHs and (c) single exposure to their oxygenated derivatives, injected separately and then mixed into the air before incubation followed by in ovo incubation of the embryonic livers on day 10 with a tritiated fatty acid mixture (palmitate 16:0). The fatty acids undergo oxidation and repetitive cleavage of carbons before entering the tricarboxylic pathway and cycles of C-oxidation. As a result of this lipid oxidation tritiated water is produced. The radioactive water (H2O) is collected and measured in a scintillator counter. Studies presenting effects on the fatty acid metabolism in avian embryos due to exposure of PAcs are absent. Using our assay the effects of PAcs and their derivatives on the enzyme function regulating the hepatic fatty acid metabolism can be studied in vitro. This approach could be a very useful trying to find out the mechanisms behind avian toxicity including disturbance of fatty acid oxidation and lipid homeostasis. To the best of our knowledge, this is the first time such a study has been performed.

We 378
Butachlor DNA binding and genotoxicity in human peripheral blood mononuclear cells
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N-(butoxymethyl)-2-chloro-2',6'-diethyl acetaldehyde, commonly known as butachlor, is a widely used herbicide in Asia, South America and Africa, for control of a range of annual and perennial grasses and some broad-leaf weeds. Extensive use of this herbicide over the years has led to the deleterious effects on non-target organisms. In this study, we have assessed genotoxic potential of butachlor in human peripheral blood mononuclear cells (PBMCs), using sensitive techniques like single cell gel electrophoresis (comet) assay, cytokinesis blocked micronucleus (CBMN) assay, and flow cytometry. Fluorescence data demonstrated significant quenching at the highest butachlor-DNA molar ratio of 1:2: The fluorescence of the staining constant (KSV), affinity constant (Ka) and number of butachlor binding sites on DNA (n) were determined to be 1.19 x 104 (r2 = 0.99), 1.2 x 104 M-1 and 1.02 (r2 = 0.99), respectively. The data revealed the binding of butachlor at minor groove of DNA. The results of comet assay showed a dose dependent increase in the size of comet tail with concomitant reduction in head size and exhibited 26-fold enhanced DNA migration. A concentration dependent increase in the total number of binuclear-micronucleated (BNMN) human PBMCs was observed upon 24 h treatment with butachlor. The data of CBMN assay indicated significantly higher (3.77 fold, p<0.05) BNMN formation. The nuclear division index (NDI) of 1.80 was estimated at 100 µM butachlor vis-a-19.12 in untreated control cells. Flow cytometric analysis of butachlor treated PBMCs showed enhancement in fluorescence of both the DCF and Rh123 probes at 25 to 100 µM butachlor due to higher ROS production. Furthermore, at butachlor concentrations of 250 and 500 µM butachlor, ROS production decreased due to substantial mitochondrial membrane damage and cell necrosis. Also, the cell cycle analysis of PI-stained butachlor treated cells exhibited an increased sub-G1 peak with concomitant reduction in G1 phase, and significant G2/M arrest, as compared with solvent control. Thus, it is concluded that butachlor treatment to PBMCs may induces oxidative stress due to intracellular ROS production, which triggers mitochondrial dysfunction and DNA damage.

We 379
National requirements in fate and behavior of plant protection products in zonal authorization in Poland
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The new - zonal authorization of plant protection product in Member States considers the submission of the dossier in accordance with proper zone: South, Central or North (Regulation 1107/2009). The requirements of fate and behavior are defined in the Regulation and in proper guidelines. For assessment of predicted environmental concentration (PEC) in particular
environmental compartment the FOCUS group has established the tools. For ground water - the PECGW calculations should be done by using of FOCUS PEARL and FOCUS PELO models. There are more than 10 scenarios, but for Poland only three are obligatory. For surface water the assessment of PECW and PECSED also should be done by tier approach using the FOCUS tools. The scenarios cover the drift, drainage and run off (D1- D6 and R1 - R4). For Poland, Central Zone, the D3, D4 and R1 are required.

WE 380

Fate of pesticides and metabolites in the Yangtze River
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The objective was to determine the fate and behavior of a model pollutant in Yangtze water sediment systems.

We investigate the fate of pesticides in Yangtze water and sediment in agriculturally influenced areas of the Three Gorges Reservoir while non-agriculturally influenced areas are used for obtaining control samples. We select propiconazole as model substance, which is known to release the priority pollutant 3, 4-dichloroaniline (DCA), DCA is also formed for our studies. DCA has been reported to form a toxic and bioaccumulative metabolite in soil and sediment, i.e., 3, 5, 4′-tetrachloroarobenzene (TCAR; H. Chisaka & P.C. Kearney 1970). We will synthesize this azo-compound by chemical means in order to study its fate. We will apply field conditions, with sediment collected in the dry season of the reservoir as well as in the temporarily flooded season, and soil at the river bank. We will incubate propiconazole and TCA in water sediment collected from the Yangtze River, applying radioactively labeled compounds in order to establish mass balances.

As preliminary studies we investigated the herbicide clodinafop-propargyl and propanil in water sediment from the river Rhine, two compounds with different physico-chemical properties. The herbicide clodinafop-propargyl is strongly adsorbed to sediment, mineralization was low, and for the non-extractable residues was also moderate. Besides, first results of the propiconazole study in water sediment systems have shown that several metabolites are formed that will be identified. By investigating the fate of a model substance known to form toxic metabolites we will address one important part of the environmental risk assessment which will be complemented by two further projects of our group on the ecotoxicity and the bioaccumulation of the pollutants.

WE 381

Comparison of the dissipation rates of an insecticide in laboratory and field studies
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Historically, laboratory-based water/sediment systems (e.g. OECD 308) have been used to estimate the dissipation rate of an agrochemical in the freshwater aquatic environment. Although these studies provide important information for the regulatory assessment of a substance, for some the dissipation rates generated may be over estimated and unrepresentative of those that are likely to be found in the real world. In the more complex laboratory and field microcosm systems, many factors can influence the fate of a substance in the water column, as well as in the sediment. We have compared the published dissipation rates of an insecticide determined in OECD Method 308 and those found in a sediment:water laboratory microcosm study, with the dissipation rates and distribution of the substance that we determined in field microcosms.

The field microcosm study was conducted in four outdoor freshwater microcosms (water depth: 30 cm) which were designed to represent the worst case of a shallow static or slow-flowing ditch. Each microcosm was established in early Spring by the addition of a clay base sediment, a layer of lake sediment (approximately 5 cm depth, 4% organic carbon) and a surface layer of aquatic macrophytes placed perpendicular to the flow axis. Continuous aeration allowed the establishment of similar and stable invertebrate communities and physico-chemical conditions. Approximately 14 days before the substance was applied, a number of aluminium trays containing further sediment were inserted into the base layer of each microcosm to aide sediment removal during the course of the study. Following the application of the insecticide, samples of water, sediment and plant material (macrophytes, and snails (Lymnea stagnalis)) were taken and analysed by liquid chromatography with tandem mass spectrometry (LCMS-MS) and the results compared to those obtained in OECD 308 and the laboratory microcosm study.

The DT50 from the water column in the field microcosm study (ca. 21 days) was approximately half of that found either in the OECD 308 study or the laboratory sediment: water microcosm (48 - 49 days). Adsorption onto plant material was found to be a major factor responsible for the initial rapid dissipation of the substance from the water column followed by adsorption and degradation in the upper layers of sediment. Our paper compares the physico-chemical properties of each test system and models the implications of the results to the design and analysis of field microcosm studies.

WE 382

Determination of environmental levels of glyphosate and AMPA in water, sediment and soil samples of the Pampas Region, Argentina
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In the last 30 years, agriculture in Argentina and other developing countries has markedly expanded. In particular, the surface of soybean crop has increased 10-fold since the 80’s. The Pampas Region is the main agriculture district of Argentina. According with current agriculture practices, Glyphosate is the major soybean herbicide. Due to the molecular multifunctional structure, this compound presents a complex environmental behavior. Its speciation and complex coordination depends on the pH, MO and free ions (Na, Mg, Ca) of the water and the redox potential of the chemical matrices demands adjustments of the analytical methods for glyphosate determination depending of the soils and water chemistry. The aim of this presentation is to report environmental levels of glyphosate, and its major metabolite the aminomethylphosphonic acid (AMPA), obtained in the last five years using methods optimized in our laboratory for the analysis of water, sediment and soil samples of the Pampas Region, Argentina. Detection method was based on HPLC-MS after alkaline derivatization using FMOC-CL. Isotopic tracers were used for quality assurance. A total number of 2.50 water samples (surface and ground water) from the region and soil samples (0-5 cm depth) were analyzed. In the first 300 samples, we found glyphosate and AMPA as major pesticides present in the environmental matrices. The highest instrumental sensitivity was obtained using ESI interface in negative mode, monitoring the M-H ion of the derivatization (glyphosate-FMOC: m/z 390, AMPA: m/z 322). Detection limits for both analytes were 0.5 µg/l for water samples and 5 µg/kg for soils and sediments. Recoveries were higher than 95% for water samples and between 85-95% for the solid matrices. In surface waters, 25% of the samples were positive with concentrations between 1-400 µg/l. None of the groundwater samples yielded positive results. Levels in soils and sediments were between 2-200 µg/kg, with 40 and 10% of the samples presenting levels above the detection limit, respectively. Optimized methods were suitable for detecting glyphosate and AMPA in different environmental matrices, demonstrating the occurrence of these compounds in surface waters, sediments and soils of the Pampas Region. On the other hand, glyphosate and AMPA, seems to not rapidly lixivate and reach groundwater.

WE 383

Distribution and degradation of triclosan in a pond mesocosm system
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The antibacterial and antifungal agent triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is a registered pesticide. But it is more frequently used in consumer products such as detergents, toothpaste, cosmetics, and antibacterial textile coatings, even though it does not provide any extra benefit to health with the exception of toothpaste (US Food and Drug Administration (FDA), 2010, [1]) and concentrations above effect levels have been measured in surface waters [2]. Triclosan is highly toxic to algae and various microbial species. Both triclosan and the metabolite methyl triclosan have a high potential to bioaccumulate in aquatic organisms [2]. The effects of triclosan on single species are well studied. However, knowledge on fate and distribution in water, sediment, and biota is scarce.

In 2011, a combined fate and effect pond mesocosm study was carried out by the German Federal Environment Agency. After single dosing of the free water, concentrations of triclosan and the transformation products methyl triclosan and 2,4-dichlorophenol were measured for 120 days in the compartments free water, sediment, aqueous, macrophytes, and snails (Lymnaea stagnalis).

Besides dissipation curves of triclosan and the metabolites in water and sediment, first results of selected measurements in biota will be presented.

WE 384

Current-use pesticides (CUPs) in surface sediments from the Bohai and Yellow Seas, North China
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With the goal of reducing human and ecosystem exposure, nine organochlorine pesticides (OCPs) included in the UNEP Stockholm Convention POPs list are eliminated or undergoing substantial reduction of consumption. The levels of these OCPs' are declining gradually in Europe, North America and the Arctic. However, some current-use pesticides (CUPs) have been observed not only in or close to agricultural areas where they are applied but also in remote areas (high mountains and the Arctic) where no CUPs have been observed not only in or close to agricultural areas.

To improve our knowledge of CUPs’ fate in the marine environment, surface sediment samples from the Bohai and Yellow Seas were analyzed for 7 CUPs (trifluralin, endosulfan, chlorpyrifos, chlorothalonil, dicrot, daclath, quintozene) and 1 metabolite (pentachlorohanoisole). Samples were collected during 2009 to 2010 using a grab sampler or a box corer, and then freeze-dried, homogenized and Soxhlet extracted for 16 hours using dichloromethane. The extracts were reduced to 2 ml using a rotary evaporator and further purified on a silica column. Elution started with 20 ml hexane (fraction 1), followed by 30 ml dichloromethane/aceton (1:1) (fraction 2), the extracts were evaporated both of fraction 1 and 2 to a final volume of 30 µL. Then all samples were measured with a GCMS-system in electron capture negative chemical ionization mode (ECNICI).

To better understand the environmental behavior of CUPs, some legacy organochlorines, such as Hexachlorobenzene (HCB) and hexachlorocyclohexanes (HCHs) were also analyzed for comparison with CUPs.

Levels, spatial distributions and possible sources of these pesticides will be discussed in this study. To better understand the environmental behavior of CUPs, some legacy organochlorines, such as Hexachlorobenzene (HCB) and hexachlorocyclohexanes (HCHs) were also analyzed for comparison with CUPs.
Fate and behaviour of the strychnin fungicide Aoxystrobin in four UK soils under laboratory conditions
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*Lancaster Universiry, Lancaster, United Kingdom
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Studies investigating the fate and behaviour of aoxystrobin in natural soils are limited. This study aimed to investigate the fate and behaviour of aoxystrobin in natural soils under laboratory aerobic conditions and for a period of 100 days. Fate and behaviour in this study were described in terms of losses due to biodegradation and extraction and remaining residues. The respective formation of non-extractable residues. Losses of the aoxystrobin were observed in all four soils and were ranged from zero to approximately 23% of the aoxystrobin associated 14C-activity. Extractability of the aoxystrobin showed to diminish with incubation time. Non-extractable 14C-residues increased with the incubation time and at the end of the incubation period more than 75% of the aoxystrobin associated 14C-activity was not extractable. Non-extractable residues were much greater than those reported from previous studies. This finding is of particular importance given that the soil used here was aged under natural environmental conditions compared to laboratory studies.

The data collected in this survey included information about routinely applied chemical products and dosages, socio-economic data and infrastructure of the studied farms. As maintaining both profitability and environmental sustainability. In this study, we performed a survey to obtain baseline information on the current status of chemical application and infrastructure of the studied farms. The study design used 3 plots per replicate, with each plot measuring approximately 1.5 m (=5 ft) wide by approximately 6 m (20 ft) long. Long duplicate blocks were as similar as possible with respect to soil and topographic parameters. Each test plot was bordered by an untreated soil area, and isolated by metal flashing. The overall slope of the tests sites ranged from plots to the parallel slope of the field. The plots were subjected to a simulated rainfall event to generate runoff (i.e., approximately 2.5 cm (1 in) of rainfall per hour). This study was conducted at multiple sites, with the treated plots at each site receiving simulated rainfall using the same simulated rainfall parameters, to provide storms of similar intensity and duration. Runoff generated during the simulated rainfall events was manually collected on timed intervals and as a complete volume of runoff.

Distribution of aged 14C-atrazine residues in soil following 22 years of environmental exposure
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Distribution and localisation of pesticide residues into the different physical fractions of soil may reveal information on processes taking place in soil. Soils amended with 14C-atrazine, were aged for 22 years under environmental conditions in a lysimeter in Germany. The soil was sampled and subjected to physical and chemical fractionation before and after incubation with the earthworm Aporrectodea caliginosa for 7 days. No significant change in the soil physical and chemical fractionation of the 14C-atrazine residues of organic carbon was observed in this study as a consequence of the activity of the A. caliginosa. The low organic carbon of the soil, the absence of relatively fresh organic matter and the long ageing time might explain the limited bioavailability of the 14C-atrazine to the earthworm. Approximately 9% of the applied 14C-atrazine associated activity was found to be present in the top soil layer 0-10 cm of the lysimeter. This amount represented approximately 36% of the total amount of 14C-atrazine associated activity in the soil after 22 years of environmental ageing. Soil half lives calculated in this study taking into account the non-extractable 14C-atrazine residues were much greater than those reported from previous studies. This finding is of particular importance given that the soil used here was aged under natural environmental conditions compared to laboratory studies.

Earthworms are used as an alternative to laboratory tests to assess the risk of chemicals in soil. However, this may not always be the case. In this study, the authors investigated the fate of aoxystrobin in laboratory experiments and in natural soils. They found that the aoxystrobin was not completely degraded in laboratory experiments, and that the non-extractable residues were much greater than those reported from previous studies. This finding is of particular importance given that the soil used here was aged under natural environmental conditions compared to laboratory studies.

Threats posed by chemical use in tilapia cage aquaculture in Southeast Asia: the case of the Moon River (Ubon Ratchathani, Thailand)
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Aquaculture is one of the most effective alternatives to meet the growing demand of food by the world population. However, modern aquaculture faces new challenges such as maintaining both profitability and environmental sustainability. In this study, we performed a survey to obtain baseline information on the current status of chemical use and aquaculture practices on 15 tilapia cage farms located in the Moon River (Maenam Mun) located in the Ubon Ratchathani Province in north-eastern Thailand. The study included 15 tilapia cage farms in the Moon River (Maenam Mun) located in the Ubon Ratchathani Province in north-eastern Thailand. The study focused on the use of antibiotics and antimicrobials in tilapia aquaculture. A total of 15 chemical products were identified as being used in tilapia aquaculture: 13 of them were related to the use of antibiotics and antimicrobials. The most commonly used group of products was the group of antibiotics, followed by the group of antimicrobials. The study found that the use of antibiotics and antimicrobials in tilapia aquaculture is a significant threat to the environment. The study also found that the use of antibiotics and antimicrobials in tilapia aquaculture is a significant threat to the environment. The study also found that the use of antibiotics and antimicrobials in tilapia aquaculture is a significant threat to the environment. The study also found that the use of antibiotics and antimicrobials in tilapia aquaculture is a significant threat to the environment.
improving the water quality of tributary; controlling activities around the reservoir and the use of bioindicator. Financing: Corumbá IV Concessões; FINATEC.

The study evaluates changes in glutathione-S-transferase (GST) and lipid peroxidation levels in the liver of Hoplobatrachus octopunctatus and B. maculatus exposed to the amendments. The analysis assessed their metabolic performance tends to be more sensitive to environmental stress and other ambient conditions. This study evaluated how whole-sediment respiration and remobilizations rates (i.e. nutrient fluxes) of urban macrobenthic communities respond to added environmental stress, such as elevated temperature, wastewater discharge and for the GST. No changes in LDH activity were noticed in the results. No significant differences were notice to the values of Ache and LPO. The response of EROD and SOD showed a diversity of responses during the time of the experiment in the site. EROD is a biomarker more sensitive due to his linkage to the genetic response of an organism to chemical pollutants, due to that is a better tool to assess the effect of the effluents. Linkages between the enzymatic effects found and parameters at higher levels of organization are easier for better understanding the mechanisms of adaptation and detoxification of molluscs in the environment.

Changes in glutathione-S-transferase levels and lipid peroxidation in Amphibians exposed to sub lethal concentrations of lead A. Enuclei1, L. Ezenyome1, I.N.E. Onwurah1, I. Tong1, T. Ogboh1

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University of Nigeria, Nsukka, Nsukka, Nigeria
National Centre for Energy and Environment, Benin city, Nigeria

The analysis tested the occurrence of histopathological lesions of different degrees of importance in liver and gills. The highest average lesion index (Index= 9.4) was observed in area associated with livestock activities. River Descoberto showed a mean lesion index equal to 8.3 to the liver and 5.3 to the gills. The historical pollution in the aquatic environment of the area analyzed was associated with the presence of the biochemical and morphological changes of the water samples with the cyano-bacteria 40cell/ml (water), the high pH (8.9-9) and BOD (35mg/L) values above the standard established by the National Environment Council - CONAMA. Among the preventive measures for the good health of fish include: improving the water quality of tributary; controlling the reservoir and the use of bioindicator. Financing: Corumbá IV Concessões; FINATEC.

Using whole-sediment mesocosm respiration rates and nutrient fluxes as a measure of community functional response to environmental stress in urban streams in Bogota, Colombia
J. Ahrens, C. Salinas
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A study was carried out on the health status of different species. Fish were collected from the place of occurrence of mortality in the reservoir toward the river Descoberto one of the main tributaries of the reservoir. Samples were collected with net and periodic inspections and fixed for taxonomic identification and light microscopy analysis. The characterization of physical and chemical (N, pH, COD, BOD and P) and microbial (cyano-bacteria) quality of water was performed to identify possible causes of histopathological lesions. The species collected were: Pimelodus maculatus, Pimelodus blochii, Leporinus friderici, Geophagus brasiliensis, Hypostomus emarginatus and Hypostomus plecostomus. The analysis performed on the incidence of histopathological lesions of different degrees of importance in liver and gills, as proliferation of melanomacrophages, necrosis, anaeurysm and neoflora. The liver damage most observed was the proliferation of melanomacrophages, present in 100% of the total sample and being used as their biomarker. Among the most serious neoplastic foci were observed in individuals of the genus Hypostomus. The highest average lesion index (Index= 8.4) was observed in area associated with livestock activities. River Descoberto showed a mean lesion index equal to 8.3 to the liver and 5.3 to the gills. The historical pollution in the aquatic environment of the area analyzed was associated with the presence of the biochemical and morphological changes of the water samples with the cyano-bacteria 40cell/ml (water), the high pH (8.9-9) and BOD (35mg/L) values above the standard established by the National Environment Council - CONAMA. Among the preventive measures for the good health of fish include: improving the water quality of tributary; controlling the reservoir and the use of bioindicator. Financing: Corumbá IV Concessões; FINATEC.

Biomarkers as indicators of stress: a case study of a tropical freshwater fish

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The reservoir of the hydroelectric plant Corumbá IV located in Corumbá River (16°24'45"S 48°10'44" W) is a complex water body located in an area with historical pollution due to chemical pollutants, due to that is a better tool to assess the effect of the effluents. Linkages between the enzymatic effects found and parameters at higher levels of organization are easier for better understanding the mechanisms of adaptation and detoxification of molluscs in the environment.

Effects of ametryn sugarcane herbicide on early-life stages and adults of a tropical fish (Danio rerio) in freshwater aquaculture


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 Glyphosate is a nonselective herbicide action, which acts against weeds. It acts by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase that acts in the synthesis of the plant’s essential amino acids essential to the plant. This work brings a study of the glyphosate interactions of glyphosate and (his) its degradation product AMPA (aminomethylphosphonic acid) with kaolinite. The analysis performed on the incidence of histopathological lesions of different degrees of importance in liver and gills, as proliferation of melanomacrophages, present in 100% of the total sample and being used as their biomarker. Among the most serious neoplastic foci were observed in individuals of the genus Hypostomus. The highest average lesion index (Index= 8.4) was observed in area associated with livestock activities. River Descoberto showed a mean lesion index equal to 8.3 to the liver and 5.3 to the gills. The historical pollution in the aquatic environment of the area analyzed was associated with the presence of the biochemical and morphological changes of the water samples with the cyano-bacteria 40cell/ml (water), the high pH (8.9-9) and BOD (35mg/L) values above the standard established by the National Environment Council - CONAMA. Among the preventive measures for the good health of fish include: improving the water quality of tributary; controlling the reservoir and the use of bioindicator. Financing: Corumbá IV Concessões; FINATEC.

Glyphosate affects the activity of acetylcholinesterase, an enzyme that regulates the hydroxyl bonding of water and the hydrogens of the octahedral surface of kaolinite.

By hypothesis, the herbicide glyphosate and its degradation product AMPA (aminomethylphosphonic acid) could be detoxifying the surface of kaolinite. The analysis performed on the incidence of histopathological lesions of different degrees of importance in liver and gills, as proliferation of melanomacrophages, present in 100% of the total sample and being used as their biomarker. Among the most serious neoplastic foci were observed in individuals of the genus Hypostomus. The highest average lesion index (Index= 8.4) was observed in area associated with livestock activities. River Descoberto showed a mean lesion index equal to 8.3 to the liver and 5.3 to the gills. The historical pollution in the aquatic environment of the area analyzed was associated with the presence of the biochemical and morphological changes of the water samples with the cyano-bacteria 40cell/ml (water), the high pH (8.9-9) and BOD (35mg/L) values above the standard established by the National Environment Council - CONAMA. Among the preventive measures for the good health of fish include: improving the water quality of tributary; controlling the reservoir and the use of bioindicator. Financing: Corumbá IV Concessões; FINATEC.

Benthic invertebrates as bioindicators of the urban impact in urban streams

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The analysis evaluated changes in glutathione-S-transferase (GST) and lipid peroxidation levels in the liver of Hoplobatrachus octopunctatus and B. maculatus exposed to the amendments. The analysis assessed their metabolic performance tends to be more sensitive to environmental stress and other ambient conditions. This study evaluated how whole-sediment respiration and remobilizations rates (i.e. nutrient fluxes) of urban macrobenthic communities respond to added environmental stress, such as elevated temperature, wastewater discharge and for the GST. No changes in LDH activity were noticed in the results. No significant differences were notice to the values of Ache and LPO. The response of EROD and SOD showed a diversity of responses during the time of the experiment in the site. EROD is a biomarker more sensitive due to his linkage to the genetic response of an organism to chemical pollutants, due to that is a better tool to assess the effect of the effluents. Linkages between the enzymatic effects found and parameters at higher levels of organization are easier for better understanding the mechanisms of adaptation and detoxification of molluscs in the environment.
To assess the efficiency of mercury trophic transfer in coastal waters off Southwest Florida, total mercury (THg) and nitrogen stable isotopes (\( ^{15}N \)) were determined in 320 coastal waters off Southwest Florida, total mercury (THg) and nitrogen stable isotopes (\( ^{15}N \)) were determined in 320

To determine the toxicity of ametryn for embryos we used the following treatments: 0 (control), 0 + (solvent control), 10.0, 16.8, 28.3, 47.6 and 80.0 mg/L ametryn. Regarding biomarkers, we observed an increase in GST and inhibition of CHOE activity in both larvae and adult fish exposed to ametryn. Furthermore, CAT and LDH activities were not significantly affected by ametryn exposure. The parameters evaluated in this study allowed a better understanding of the mode of action and toxicity of ametryn in fish species.

In this contribution, the toxicity and accumulation of two non-essential metals (cadmium and lead) to the tropical snail Archachatina papyracea were assessed. As far as we know, this is the first study assessing metal toxicity to this tropical snail species. Specimens of the snail A. papyracea were exposed in a natural soil collected from Ilhe, Nigeria and spiked with varying concentrations of Cd and Pb over a 28-day period. Survival and weight change of snails were monitored weekly while tissue accumulation was assessed at the end of the 28-day period. Survival was a more sensitive endpoint than weight change of snails. The LC50 (effect concentration at which 50% of snails died) value (with confidence interval, CI) for Cd was 87 (75-100) mg/kg while the EC50 value (with CI) for weight change was higher at 4159 (3033-5702) mg/kg. Therefore, Cd was a factor of 4.6 times more toxic than Pb to the snail A. papyracea.

In this contribution, the toxicity and accumulation of two non-essential metals (cadmium and lead) to the tropical snail Archachatina papyracea were assessed. As far as we know, this is the first study assessing metal toxicity to this tropical snail species. Specimens of the snail A. papyracea were exposed in a natural soil collected from Ilhe, Nigeria and spiked with varying concentrations of Cd and Pb over a 28-day period. Survival and weight change of snails were monitored weekly while tissue accumulation was assessed at the end of the 28-day period. Survival was a more sensitive endpoint than weight change of snails. The LC50 (effect concentration at which 50% of snails died) value (with confidence interval, CI) for Cd was 87 (75-100) mg/kg while the EC50 value (with CI) for weight change was higher at 4159 (3033-5702) mg/kg. Therefore, Cd was a factor of 4.6 times more toxic than Pb to the snail A. papyracea.
Influence of the pH on the acute toxicity of vinasse to microcrustaceans
R.G. Botelho, L. Maranho, L. Machado Neto, V. Tornisielo Center for Nuclear Energy in Agriculture, Piracicaba, Brazil Piracicaba (State of São Paulo) is a major producer of sugarcane in Brazil. Vinasse is generated in the process of alcohol distillation, and is composed of water, nutrients such as nitrate, potassium, high organic matter and acid pH. These characteristics make the vinasse have high pollutant power, which is about one hundred times greater than that of domestic sewage, characterizing it as harmful to the animal and plants. This residue has been used in the fertilization of sugarcane due to its composition rich in nutrients; however, when applied in high concentrations can contaminate aquatic environments through leaching and runoff. The aim of this study was to evaluate the chronic toxicity of vinasse to the microcrustaceans C. dubia and C. silvestrii. All concentrations were performed in triplicate in 1 L aquariums and the experiment was conducted for 14 days. The pH study showed that the acid pH of the vinasse can be corrected to achieve LD100 of 0.45%. After pH correction, the chronic LD50 for both species was calculated using the statistical program TRIMMED SPSS. The pH study revealed that the vinasse has high acute toxicity, and corrective measures have been proposed to reduce its impact on aquatic ecosystems.

Acute toxicity of the vinasse on Danio rerio
V.L. Tornisielo, R.G. Botelho, L. Machado Neto, L.A. Maranho Center for Nuclear Energy in Agriculture, Piracicaba, Brazil Danio rerio (Philadelphia eel) is a majior producer of sugarcane in Brazil. Vinasse is generated in the process of alcohol distillation, and is composed of water, nutrients such as nitrate, potassium, high organic matter and acid pH. These characteristics make the vinasse have high pollutant power, which is about one hundred times greater than that of domestic sewage, characterizing it as harmful to the animal and plants. This residue has been used in the fertilization of sugarcane due to its composition rich in nutrients; however, when applied in high concentrations can contaminate aquatic environments through leaching and runoff. The aim of this study was to evaluate the chronic toxicity of vinasse to the microcrustaceans C. dubia and C. silvestrii. All concentrations were performed in triplicate in 1 L aquariums and the experiment was conducted for 14 days. The pH study showed that the acid pH of the vinasse can be corrected to achieve LD100 of 0.45%. After pH correction, the chronic LD50 for both species was calculated using the statistical program TRIMMED SPSS. The pH study revealed that the vinasse has high acute toxicity, and corrective measures have been proposed to reduce its impact on aquatic ecosystems.

Use of pesticides at Bom Repouso, Brazil, and soil risk assessment
M.E.T. Nunes, E. Espindola, I.K. Rodrigues, C.D.L. Lima, A.V. Wachslitz 1Escuela de Engenharia de São Carlos/Universidade de São Paulo, São Carlos, São Paulo, 2Departamento de Biologia/Universidade Federal do Amazonas, Manaus, Brazil The district of Bom Repouso, Minas Gerais State, Brazil has their economy based on agriculture, with potatoes and strawberry as the main crops. The widespread use of pesticides and other agrochemicals is common. This work aimed to evaluate the environmental impact of the use of pesticides on the soil. The environmental impact was assessed through the calculation of the Toxicity Exposure Ratio (TER) by dividing the LC50 (14 days) for earthworms, obtained through standard ecotoxicological tests adapted to tropical conditions by the respective Predicted Environmental Concentration (PEC), considering soil properties, pesticide chemical properties and application rates. The PEC was calculated using the FOCUS model. In three scenarios that considered actual rates of application of the product, based on information provided by farmers, potential chronic risk to soil organisms was identified. The data obtained in this study are important for future ecological risk assessments in tropical regions.
runoff water from the uncontaminated soil (UNR); runoff with runoff soil sprayed with Vertimec® (CR) and direct application of Vertimec® (V). For this purpose, two parcels of soil (8m2) were used: one control and another one contaminated with Vertimec (dose recommended for strawberry cultures). After that, a typical rain in tropical systems was simulated (20-30mm) and the water from the runoff was collected in the mesocosms (UNR and CR). Besides, water samples were collected on the mesocosms after the input of runoff to evaluate and compare the results in both experiments. Water and sediment samples were also collected for physical and chemical analysis. The limnological analysis showed the increase of nutrients (nitrogen and phosphorus), suspended solids, turbidity, chlorophyll and conductivity. On the other hand, reduction in dissolved oxygen concentration was observed in UNR and CR after runoff addition. In relation to bioassays, chronic toxicity was registered only in the in situ experiment, with considerable effects on the survival and biomass.

### WE 411

#### Hematological biomarkers in tadpoles of Lithobates catesbeianus exposed to agrochemicals mixture in rice crop


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The pesticides to chemicals and to rice crops and water is recommended to improve yield. However, their use may affect non-target organisms, as the amphibians. Blood is an excellent tool for assessing the physiological, biochemical and pathological conditions in animals, because it’s in contact with tissues, organs and cells and react sensibly to all the changes that take place. The aim of this study was to evaluate the potential toxicity to tadpoles of L. catesbeianus in rice crop treated with agrochemicals mixture and determine hematological parameters. The compound chosen to realize the tests is a commercial rice crop treated in the state of São Paulo, Brazil. Before 25 days of applying pre-germinated seeds, previously treated with Fipronil, the court was drained for the application of the pesticides: Fenoxaprop 240g/L (150mla) + vegetable oil (1Lha) and Mefentraox 50g/L (150ml/kg). After 3 days of application, the court was dried and 60 hours after, the exposition was applied Carbosulfan 50g/kg (500g/ha) with the fertilizer. Gently caging measurements 0.8x0.8x0.4m were fixed inside the rice crops, allowing the tadpoles to access the sediment. In each group it was used 150 tadpoles in the 3 Gosner stage (60) with medium weight about 0.64g. Before the beginning of the tests and in the experimental days 3, 7, 10, 14 and 21 it was take 6 animals/group. These animals were anesthetized to blood collection to evaluate hematological parameters: hematócrit (Ht), hemoglobine tax (Hb), counting of erytrocyte’s number, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). After 21 days of exposure, it was verified the survival tax 100% in the control group, 92.7% in the exposure group. The concentration of Ht, Hb, MCV and MCHC don’t show significant differences: (Ht) 19.37 ± 2.29%, (Hb) 21.10 ± 4.74 ± 104/mm3, (MCHC) 105± 20 γ/dl with medium significant difference only in the 14th days, medium values of 4.13 ± 0.70g/100mL to control group and 2.74 ± 0.62g/100mL to exposure group. During the others analyzed days the Hb was keep in normal values of 3.5 ± 0.37g/100mL. The tadpoles of L. catesbeianus show resistance to agrochemicals used in rice crops observing the small mortality.

### WE 413

#### DNA Damage in childish fish and environmental forensic analysis of the origin of PAHs at a Guatemalan oil field

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**Texas A&M University, College station, United States of America**
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The oil industry is a large-scale producer of polycyclic aromatic hydrocarbons (PAHs). Cichlid fish (Tanganichus meeki and Vieja synspila) were collected for determination of DNA strand breakage (by gel electrophoresis) and chromosomal breakage (flow cytometry). PAHs in water and sediment were extracted with methylene chloride and analyzed by GC/MS. Environmental forensic analysis was also carried out using three metrics to draw inferences about the origin (pyrogenic, petrogenic, diagenic) of the PAHs in the sediment. These metrics included a) concentration (mg/kg) of PAHs with 4 or more rings, b) a “pyrogenic index” defined by as [all other 3-6 ring PAHs]/kalkylated [naphthalenes, phenanthrenes, dibenzothiophenes, fluorines, and chrysenes], and C) ratio of chrysenes/phenanthrenes in sediments. Multivariate analyses were also used to analyze all three of these variates together. For T. meeki from Xan field, chromosomal breakage and strand breakage was greater than in the control group and in Vieja field, chromosomal breakage and strand breakage was greater in Xan than one of the two reference sites. Patterns of aqueous PAH concentrations, suggest that fish are affected by anthropogenic contaminants, which are elevated at some reference sites, but environmental forensic analysis suggested a pyrogenic or diagenic origin. The environmental forensic analysis also suggested that PAHs near the oil well injection site were primarily of petrogenic origin, and the ratio of aromatic/phytanic PAHs decreased with distance from the oil field. The data are consistent with the hypothesis that oil field brines injected into the ground water caused genotoxicity in fish at Xan field, and it is also possible that pyrogenic PAHs influence levels of DNA damage in reference sites. These analyses represent one of the first efforts to examine genotoxicity in native Mesoamerican cichlids.

### WE 414

#### Effects of trichlorfon on the neotropical freshwater fish tambaqui, Colossoma macropomum

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The use of pesticides has been the main strategy to prevent ectoparasites that threaten acuitre culture productivity. The non regulated use of such chemicals may lead to damage in aquatic ecosystems, causing the ponds ecosystem imbalance and costs, and in an alternative to reduce the economic losses associated with pesticides. These chemotherapeutic agents actually constitute one of the main environmental problems derived from intensive fish culture. Trichlorfon (O-O-dimethyl O-2,2,2-trichloroethoxy) is one of the most used products against fish parasites in aquaculture. In normal conditions of use, it is very quickly hydrolyzed to trichloroacetic acid which is much more toxic. This study examined biochemical alterations (serum and plasma) in neotropical fish tambaqui, Colossoma macropomum (Wt: 49,1 g) exposed to a concentration sublethal trichlorfon (0,125 mg/L, trichlorfon 97/100g). The experimental design was randomized with three replicates in a semi-static. During the experiment, the fish were not fed. AST, ALT, DLH and Glucose analyzed were spectrophotometrically (enzyme kit). The cortisol determined with kit (19.41 ± 3.54 ng/dl) was again flooded and the tadpoles were exposed. And after five days about the beginning of the exposition was applied Carbofuran 50g/kg (500g/ha) with the fertilizer. Hb shows significant difference only in the 14th day, medium values of 4.39 ± 0.70g/100mL to control group and 2.74 ± 0.62g/100mL to exposure group. During the others analyzed days the Hb was keep in normal values of 3.5 ± 0.37g/100mL. The tadpoles of L. catesbeianus show resistance to agrochemicals used in rice crops observing the small mortality.

### WE 415

#### Urban geochemistry of the Berlin Metropolitan Area, Germany

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A third of the population of Germany lives in cities with a population greater than 100,000. Anthropogenic activities are the determining ecological factor in cities and...
industrial regions. Berlin is one of the first European megacity to be covered in its entirety by a geochemical survey of topsoil, including large, more or less rural areas. The regional or local geochemistry of an urban area can be evaluated only by comparison with the non-industrial surrounding area.

The distribution of 43 trace and 9 major elements in 3,746 topsoil samples (0 - 20 cm) in Berlin and surroundings was mapped and interpreted. Studies of the Berlin metropolitan area and other urban regions have shown that heavy metals (e.g., Cd, Cu, Cr, Hg, Ni, Pb, Zn and As) are enriched in the upper parts of the urban landscape, with concentrations increasing with distance from the city center. High concentrations of metals (especially lead) and polycyclic aromatic hydrocarbons (PAH) are found in urban topsoil, resulting in an accumulation of pollution in the urban area. The sunscreen contains the same or similar chemicals such as Everything, consisting of organic compounds such as toluene, xylene, and ethylbenzene, which are used in its manufacture and purification processes. These chemicals can be harmful to the environment and human health if not properly managed.

In the present study, the presence of hazardous organic chemicals in recycle tire playground surfaces is investigated. With this purpose, direct material analyses using solvent extraction as well as SMPE analysis of the vapor phase above the samples are carried out. This study provides a comprehensive characterization of the organic compounds present in recycle tire playground surfaces, which can be used for risk assessment and remediation purposes.

The 2010-2011 study was carried out to evaluate whether the German Federal Water Act (GFU) can be used to judge the suitability of a given water body as a source for drinking water. The study compared two water bodies: the Meuse River and the Rhine River. The Meuse River was chosen because it is a main river in the Netherlands and is used as a source for drinking water. The Rhine River was chosen because it is one of the most heavily polluted rivers in Europe and is used as a source for drinking water in several countries, including Germany.

This study was carried out to evaluate the suitability of the Meuse River and the Rhine River as sources for drinking water, based on the results of the 2010-2011 study. The study found that the Meuse River was more suitable than the Rhine River, due to lower concentrations of pollutants and higher water quality indicators. The study also highlighted the importance of monitoring water quality in urban areas, including the presence of hazardous organic chemicals in recycle tire playground surfaces.

The Dutch drinking water company Dunea uses water from the river Meuse as source for its drinking water production. Yearly 80 million m3 surface water is abstracted for this purpose. The company has been managing the water quality of the river Meuse for many years, using various monitoring techniques and methods. The company has also been collaborating with the Dutch Water Laboratory (WCA) and the University of Delft to improve the monitoring and management of the water quality of the river Meuse.

The monitoring had resulted in a wealth of data since some of the water quality parameters had been monitored up to 52 times a year. A proper comparison was however challenged by the fact that monitoring programs on the two rivers were different and changed over time. To account for these problems, different groups of chemicals were identified for comparison with the non-industrial surrounding area. Both single-element and multi-element maps (cluster-Q analysis, principal component analysis), and maps showing the geochemical load index for various trace elements were used to evaluate and interpret geochemical data. Study of regional variations and anthropogenic contamination of topsoil by metals and organic pollutants is important for environmental planning and monitoring in urban areas. A geochemical survey of the urban environment provides a reliable database for setting concentration limits for urban and other soils. Sustainable urban development requires combined management and monitoring of urban soils and chemical fluxes. Geostatistics and multivariate statistical methods provide a comprehensive understanding of the spatial distribution of urban pollution and alteration of soils.

For example, a study was conducted in Shanghai to assess the health effects of heavy metals in urban soils. The study found that the levels of heavy metals in urban soils were higher than the regulatory limits set by the Chinese government. This indicates a need for increased monitoring and management of urban soils to ensure the safety of the urban environment.

This study is the first comprehensive study to assess the health effects of heavy metals in urban soils in Shanghai. The study was carried out by the Shanghai Environmental Protection Bureau and the Shanghai Institute of Environmental Science.

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for drinking water preparation. In case of a calamity at the River Meuse, Dunea thus could decide to temporarily use the River Lek instead. The study furthermore provided results of the PM$_{10}$ air samples indicated an average tread concentration of 0.12 µg/m$^3$, contributing 0.62% to the overall PM$_{10}$ in a coastal environment subjected to chronic pollution by pyrolytic PAHs, episodes of petrogenic pollution, like oil-spills, can be identified by the combination of different source ratios. Results also indicate that in the study area PAHs are transported from superficial water to sediment. This conclusion is based on the degree of coincidence found in the occurrence of individual PAHs in both compartments and in their petrogenic/pyrolytic nature; the positive sedimentation rate in the study area together with the performance of the analyses on unfiltered water; and the distribution of sources of PAHs found in the area.

WE 427

Evaluation of tire and road wear particles in the Seine River watershed: a sustainability initiative of the global tire industry

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The Tire Industry Project (TIP), organized as a sector project under the World Business Council for Sustainable Development, has been engaged in a multi-year project to understand the potential for environmental health risk associated with tire and road wear particles (TRWP). In order to understand the potential for exposure to TRWP, a global comprehensive chemical and biological monitoring study (INRA) was implemented in the Seine River watershed to quantify levels TRWP in air and sediment at sites representative of potential human and ecological exposure. The Seine River watershed was selected from among four watersheds in Europe based on water quality criteria, a representative range of PM$_{10}$ levels, and size and diversity of the watershed. Sampling sites were selected to represent a wide diversity of human receptors and ecological habitats. For all media, the presence of an identifiable vehicle traffic source was mandatory with additional consideration given to sample point accessibility, available sampling, data, site description, and land use. A chemical marker specific to the polymer portion of TRWP was used to quantify the spread of material within the watershed. Results from the INRA study show that there was a trend of organic and inorganic contaminants in the sediments of the port area. Unlike for the Port of Aveiro, results from Portimão port reveal that there was a trend of organic and inorganic contaminants in the sediments of the port area. For all media, the presence of an identifiable vehicle traffic source was mandatory with additional consideration given to sample point accessibility, available sampling, data, site description, and land use. A chemical marker specific to the polymer portion of TRWP was used to quantify the spread of material within the watershed. Results from the INRA study show that there was a trend of organic and inorganic contaminants in the sediments of the port area. Unlike for the Port of Aveiro, results from Portimão port reveal that there was a trend of organic and inorganic contaminants in the sediments of the port area. For all media, the presence of an identifiable vehicle traffic source was mandatory with additional consideration given to sample point accessibility, available sampling, data, site description, and land use. A chemical marker specific to the polymer portion of TRWP was used to quantify the spread of material within the watershed.
Acetylcholinesterase (AChE), superoxide dismutase (SOD) analysed in the gills of Mytilus galloprovincialis from the same area revealed that AChE was inversely related with Zn levels in sediments while SOD increased. There was, however, a direct relationship between Cr in the sediments and catalase level in mussels the digestive gland while in the gills lipid peroxidation increases.

The results revealed the need to analyze all these descriptors at the same time as proposed by the Water Framework Directive and the Marine Strategic Framework Directive to assess to the good ecological status in Port areas.

**WE 430**

Trend analysis of ambient lead levels in water, air, sediment and soil samples

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Due to the reduction of diffuse emissions of lead into the environment over the past decades (e.g., restriction of lead addition to fuel), it is expected that ambient lead levels in different environmental compartments (water, sediments and soil) have decreased over time. The evolution of lead levels in these compartments, using available monitoring data, was investigated and long-term data series (1980 through 2011) were analysed.

National authorities were contacted to provide monitoring data for the water, air, sediment and soil compartment. Additionally, publically available datasets were retrieved from the OECD database, US EPA, Germany, UK, Ireland, France and Sweden provided good quality data. Leading edge concentrations in water and sediment. All datasets were evaluated qualitatively and approached statistically to derive trends in lead concentrations. Air concentrations were taken from the European Monitoring and Evaluation Programme (EMEP), scientifically based and policy driven programme under the convention on long-range transport of air pollution.

The analysis of the collect monitoring data showed that Pb emissions in the environment have generally decreased due National and European emission reduction strategies.

Reduction of Pb emissions appeared to have a direct impact on the water (both total and dissolved levels) and air compartments, where a clear decreasing trend was spotted. Pb concentrations in some lakes, however, were not fully in line with these findings, most likely related to the specific nature of a lake environment, where identifying a trend is more complex as this is much more dependent on geo-chemical characteristics of the area combined with human activities.

Based on the retrieved and processed data on terrestrial and sedimentary compartments, a decreasing trend was also identified.

**WE 431**

The trend of PRTR data and air toxics monitoring data in Japan

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In Japan, the PRTR Law was implemented in 2000 and the compiled data is available by all the people. This dataset gives various information on the status of pollutant emissions. Regarding monitoring data, the air toxics monitoring campaign by Ministry of Environment has started in 1997. This campaign covers all over Japan whose number of monitoring sites is around 300.
The first objective of this study is to clarify the year-by-year trend of PRTR data and air toxics monitoring data through 2000 to 2009. The second objective is to analyze the relationship between PRTR data and monitoring data. This paper, following previous papers by authors (Katatani et al., 2006 & 2007), reports some results of trend analyses and statistical analyses. The analyses were carried out on the prefecture-by-prefecture basis.

The principal results are as follows:

1. Air pollution by toxic chemicals in Japan are generally decreasing except some specific species. It can be considered that they are partly resulted by governmental regulations, and partly resulted by self management by many industries. 2. The consistency between PRTR data and air monitoring data varies

**WE 432**

Assessment heavy metals pollution of snow cover using chemical and ecotoxicology methods

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The snow cover accumulates in its composition practically all pollutant emissions in atmosphere. For study are chose 10 crossroads and 20 streets in Kiev (city in Russia Federation 58° 36’ 0” N, 49° 39’ 0” E). Tests of the snow were collected under stable weather conditions during winter period (December 2010 - march 2011) in 30 sampling points, samples were taken five times at each point.

The studies included pH, salinity, heavy metals, (Cu, Cd, Pb, Zn and Ni), bioassay. The bioassays evaluated were 1) E. coli (with gene-modified luciferase) - bioluminescence, 30-min. 2) Higher plant S. alfa - seed germination, growth rough 4-day, 3) B. taurius (sperm cells)-mobility, 3 h.

According to the results of the experiment revealed patterns of distribution of heavy metals in the snow. By bioassay evaluated the toxicity of snow. A comparison of data of chemical analysis and bioassay. 

The Designed method for assessing the spatial distribution of pollutants emissions heavy metals from motor transport in atmosphere in urban environments in snow cover.

**WE 433**

Contamination status and spatial distribution of persistent chlorinated and brominated organic contaminants in the European eel (Anguilla anguilla) in Flanders, Belgium

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Pooled yellow eel (Anguilla anguilla (L.)) samples, consisting of 5-10 eels, collected between 2000 and 2009 from 60 locations in Flanders (Belgium) were investigated for their content of lipophilic and persistent contaminants such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs).

Eel is a fatty and sedentary fish species, assuring a high accumulation of lipophilic contaminants and providing a representative estimate of contamination patterns within the catching area. European eel stocks are in decline in most of their geographical distribution and their status is considered below safe biological limits. A variety of contaminants is known to affect the eel and the eels were expected to be present on several levels of biological organization, including population level. The aims of the present study were to investigate the current PCB, PBDE and HBCD contamination levels in wild eels throughout a bio-monitoring network in the freshwater system in Flanders, Belgium.

The current study expands the knowledge regarding these contaminant concentrations, their patterns, distribution profiles and time trends in aquatic ecosystems. PCBs, PBDEs and HBCDs concentrations were detected in all analyzed eel samples and some samples had high concentrations (up to 41600, 1400, 9500 ng/g lipid wt., respectively). CB-153 was the most dominant PCB congener, closely followed by CB-138 and CB-149. Among PBDEs, BDE-47, -100 and -99 were the predominant congeners, similar to the composition reported in the literature. For HBCDs, α-HBCD was predominant followed by ω- and β-isomers in almost all eel samples. The broad range in PCB, PBDE and HBCD concentrations reported in the current study is likely due to the variety in sampling locations, from highly industrialized areas to small rural creeks. PCB levels accounted for the majority of the contamination in most samples. The contribution to the total human exposure through local wild eel consumption was also highly variable.

Some eels (16 sites) exceeded largely the new EU consumption threshold for PCBs (300 ng/g ww for the sum 6 indicator PCBs: CB-28, -52, -101, -138, -153 and -180). The current data show an ongoing exposure of Flemish eels to PCBs, PBDEs and HBCDs through indirect release from sediments or direct releases from various industries.

Therefore concerns are raised regarding the impact of these contaminants on eels and on the human exposure close to industrialized hotspots.

**WE 434**

Mercury levels in food from the Idrija mercury mine area

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Due to the novelty of sampling and ministering activities (1508-1994), the soil in the Idrija region is highly contaminated with mercury. In order to check the present situation regarding the contents of mercury in selected local food samples, levels of total mercury (THg) and methyl mercury (MeHg) were determined in foodstuffs, mushrooms, fish and deer from the Idrija mercury mine area. THg in freeze dried foodstuffs and in freeze dried mushrooms was determined by thermal combustion, amalgamation and atomic absorption spectrometry using a Direct Mercury Analyser (DMA-80). THg in fish was determined by chemical digestion and cold-vapour atomic absorption spectrometry (CVAS). MeHg in freeze dried foodstuffs was determined by cold vapour atomic fluorescence spectrometry (CVAFS) using Brooks Rand MERX -Automated Modular Mercury System. MeHg in fish samples was determined by Tetramethyl ammonium Hydroxide (TMAH) extraction followed by aqueous phase extraction, GC and CVAFS detection using Brooks Rand MERX -Automated Modular Mercury System. Mercury levels in analysed food were not very high but were elevated compared to the levels in food from the non-contaminated areas. However, our study showed that THg is accumulating in mushrooms (X=379 ng/g wet weight, Med=113 ng/g wet weight) and chicory (X=727 ng/g wet weight, Med=173 ng/g wet weight). Based on previous data available, we can conclude that the levels of mercury in food have not diminished significantly during the past 15 years after the closure of mercury mine. Further work will include the study of the influence of elevated levels of mercury in foodstuffs on the content of metals such as selenium, cadmium and lead.

**WE 435**

Pooled polybrominated diphenyl ethers (PBDEs) in Chinese electrical waste recycling areas: human exposure and policy strategies

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Polybrominated diphenyl ethers (PBDEs) are brominated flame retardants (BFRs) widely used in numerous polymer-based commercial and household products. Electrical appliances are thought to be the major source for PBDEs in China. China plays a key role in the electronics industry, producing a significant share of the sector’s worldwide output. Similarly, electronic waste (e-waste) has become a major environmental concern in China because of the potential detrimental impacts on the environment caused by the crude processing used in e-waste dismantling and associated release of toxic chemicals such as PBDEs. PBDEs are often exported to China as e-waste, second-hand electronics, and used automobiles. Therefore, e-wastes are an important source of environmental contamination by PBDEs in China.

SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
In this study, we gave a comprehensive report on human exposure to PBDEs in and around Chinese e-waste areas. Based on the result, we put forward current existing limitations of current assessment methodologies, regarding the evaluation of impacts in a world where complex problems exist, result in a need for a thinking that goes beyond one of ‘deduction’ alone.

The Authors are working on the definitions of new features that can improve LCA tool in order to develop it from E-LCA to Sustainability LCA.

Beyond environmental assessment tool to design with systemic methodology

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A Conceptual Vision of Sustainable Design may provide new perspectives for problems that cannot be easily addressed within a single discipline. On the basis that living systems are open, they continually draw upon external sources of energy and maintain a stable state of low entropy, as the physicist Erwin Schrödinger asserted in 1946; some of the next theories on industrial processes applied that concept also on artifical systems. Material and energy loops are open in order to decrease environmental impacts and resources flows in the system. The method that can assess resource depletions of the all metal and rare earth are developed. LIME (user cost) with ReCPe (surplus cost) are compared to pick out the problem. And, a new method using the concept of shadow price are developed to assess the reserve, grade, cost of mining, market price for the rare metals.

Environmental LCA is one of the most internationally recognized methodology for the environmental evaluation, related to a product or a service during all of the life cycle stages. Nevertheless the life cycle assessment approach is based on a linear model of a real process and the evaluation of the environmental burdens is a little bit partial. The limitations of current assessment methodologies, regarding the evaluation of impacts in a world where complex problems exist, result in a need for a thinking that goes beyond one of ‘deduction’ alone.

The Authors are working on the definitions of new features that can improve LCA tool in order to develop it from E-LCA to Sustainability LCA.

Sustainability LCA not only provide the environmental impacts of different materials, but also is able to suggest new use of material and energy flow strictly connected to local resources, biodiversity, human welfare, etc.
Communication of LCA results: how to support decision making
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In order for an LCA to fulfill its role in effective decision support, the output indicators should address the specific needs of the decision to be supported and should be communicated to the decision maker. In actively there is therefore a need to define 1) what are the different decision contexts and 2) the types of decision criteria for the different contexts. Bearing in mind that the criteria probably change with each case study the decision maker have to, based on these criteria, weigh a) among different environmental impact categories as well as b) among impacts from all sustainability pillars. Which of the LCA results are more important for the decision maker and how does the technological geographical and temporal contexts of the specific case study affect the decision in question? Can archetypical decision contexts and criteria be identified? What are possible alternatives to weigh among the different environmental impact categories and prioritise the alternative decisions? How to integrate in the decision process different metrics for social and economic impacts and what are the alternative ways for result communication? These questions will be addressed in an attempt to identify sustainability assessment output results that could maximise the applicability of LCA without overwhelming and/or confusing the decision maker. The field of application are existing LCA case studies from the industrial sector.

Agent-based Modelling of the agricultural industry sustainability of the Picardie/champagne region (France)
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Attaining industrial sustainability at the local/regional level requires collaborative efforts from participating agents toward common goals including resource conservation, low carbon emission, production efficiency, economic viability, and corporate social responsibility. Our existing socio-technical systems should transition or evolve towards achieving system’s sustainability. This study aims to operationalize the idea of system’s sustainability by developing an Agent-based model framework for designing an industrial ecology-modeled agricultural bioenergy firm. Agricultural production and its management is seen as an evolving system of complex interactions between nature, physical structure, market rules, and participants. Participants face risk and volatility as they pursue their goals and make decisions based on limited information and their mental model of how they believe the system operates. The purpose of simulation modeling such as Agent-based modeling is to generate and explore alternative futures that may develop under different conditions. These simulations can explore various “what if” scenarios under different economic, social goals. They can show the possible evolutionary trajectories of given scenarios under different conditions and geographical context. An industrial eco-park organized around the agricultural product industry is a relevant area of application: On one hand, this task will identify and produce a first contextual analysis of the structuring factors and the main driving forces for the development, along the way, of prospective scenarios (economic constraints, regulations, environment, and political context; carbon market; technological routes, etc). On the other hand, the goal will be to confront visions resulting from the possibilities of realization of the future with the models studied in task number one. Indeed, the industry due to its current economic challenges and inherent structure offer a variety of opportunities for synergies. For this model the actors (agents) will be agricultural-based food plants, bioenergy plants, and agricultural. The goal of this agent-based model is to see how they can work in symbiosis both for the conditions for them to allow transfer of flow of material between their plants, the main forces will their sensitivity to regulation of Co2 emissions, and subsidies incentives.

Diverging technology rankings in the NEEDS project: analysing the compatibility of the total cost and MCDM approaches
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In the European project New Energy Externalities Development for Sustainability (NEEDS), two different kinds of approaches were used to rank power generation technologies: (i) the costs (techno-economic externalities) and (ii) Multi-Criteria Decision Analysis (MCDM). Both approaches made use of Life Cycle Assessment (LCA) indicators. In the end, they yielded rather different technology rankings that can be explained by differences in concept, quantification and scope. The aim of this study is to identify to what extent these ranking methods are different as a matter of principle and to what extent the ranking approaches used in NEEDS can be modified so as to cover a similar range of aspects.

The study identified that there is a considerable degree of overlap between the approaches MCDM and total costs. However, crucial differences exist that would prevent the rankings from becoming equivalent despite potential efforts for their harmonization. The following main reasons for this conclusion were identified: (i) MCDM does not require ranking to be expressed in monetary terms; (ii) In the consideration of social, environmental, and economic aspects in MCDM is not limited to private costs and (technological) external costs as is the case for the total cost approach. In NEEDS, most of the technological externalities that were covered in the MCDM were also covered in the total cost approach. The main except in concerns biodiversity-related externalities, followed by noise-related impacts, visual impacts on landscapes and risks associated with waste storage. Further indications considered in the NEEDS MCDM are mainly beyond the scope of the total cost approach. If these aspects were important in decision-making, efforts aim at harmonizing the total cost and MCDM approaches would be rather useless. The two approaches also differ also in how risk aversion is taken into account. (iii) Individual preferences have a strong influence on the MCDM rankings. This is because they can put higher or lower weights on "hard indicators" like private costs.

In the end, any analysis seeking to comprehensively evaluate external costs will be limited due to the constrained possibilities to monetize externalities, which mostly concern biodiversity or ecosystem services-related externalities. Even though generally available, yet more research is needed in this domain.

Subcategory assessment method (SAM) for S-LCA consumer stakeholder: method and application on a family Italian winery
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Going from raw data to impact assessment in S-LCA is still a challenge. Over the years, several methods have been presented in the literature. Due to the limits of the methods related to subcategories listed by UNEP and SETAC (2009), the objective of this paper is the proposition of a Subcategory Assessment Method (SAM) to reduce the variability of the evaluation of subcategories in S-LCA studies. A proposal for stakeholder consumers and a case study are presented. SAM includes 3 subcategories (health and safety, ecological impact, and social aspects) for the stakeholder consumers and enables analyzing the organization in a Likert scale: A, B, C, D. Filling Class B means that the organization meets basic requirements which are defined for each subcategory, based on International Agreements. Class A means that the organization shows more proactive behavior than established in basic requirements. Classes C and D are below basic requirements and are differentiated due to generic data (may be information about the country or sector). Afterwards, SAM was applied in a small winery in Italy. The goal of the case study was to analyse the wine consumer’s subcategory. The functional unit considered was the production of a bottle of wine (‘novello’ produced and consumed in the same year). Specific data (data from the site) was collected by interviewing managers and technicians of the wine company and representatives of consumer organizations. A questionnaire was prepared, based on the methodological sheets proposed by UNEP and SETAC (2010), and sent to the wine company and the consumer organizations. The results showed that the company did not reach the basic requirement for most of the subcategories. However, in some cases, simple behavior changes would improve the performance. The case study also showed that it was possible to collect data to and evaluate the company regarding the consumer stakeholders using SAM. The method could also be implemented for the entire product life cycle. On the other hand, one should be aware that SAM is as time and work demanding as (environmental) LCA, because the information changes from company to company, sector to sector and region to region. Future development of SAM could include the remaining subcategories, providing basic requirements for each of them.

LCA of ‘drop-in’ biofuel supply chain: case of UMaine TDO advanced biofuels
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To date, there has not been a clear definition of the term “drop-in” biofuels. Literature consulted use “drop-in biofuels” interchangeably with the term “advanced biofuels”. This is visible in the naming of research consortiums working on achieving the goals of replacing conventional fuels directly with bio crude, bio gasoline and bio jet fuel. Examples can be seen in the UMaine Technology Development Organization (TDO) of the United States (made up of 17 partners from industries, universities and national laboratory). It was formed recently to oversee the development of direct replacement biofuels or supplements to existing gasoline, diesel and jet fuel that could be used without making changes to existing fuel distribution networks or engines. The other important example mentioned is the formation of the French Advanced Biofuels (ABF). With the movement towards ensuring sustainability of the earth’s resources gaining ground, Life cycle assessment (LCA) which is gradually being reformed into Life Cycle Sustainability Assessment studies as an impact assessment approach is on the rise. There have been numerous LCA studies in various fields of endeavors and this has even led to the modification of LCA from an environmental profiling tool to a sustainability measurement tool in the form of the UNEP developed Life Cycle Sustainability Assessment (LCSA) framework which will shed light on the LCA studies that have been conducted on drop-in biofuels over years and will elaborate on the key characteristics of the studies conducted in this field.

The second part of the presentation will deal with the Life Cycle Sustainability Assessment procedure developed by the Research Group for Industrial Ecology, LCA and System Sustainability (HEL CAS) at the University of Twente. This procedure which integrates concepts such as system dynamics, agent based modeling, multi criteria decision making analysis, input-output analysis and GIS with LCSA perspective will help us analyze the sustainability of biofuels supply chains.
WE 449
Life cycle sustainability assessment of ethanol production using the virtual sugarcane biorefinery framework
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The sugar cane virtual biorefinery (VSBI) is an innovative framework that integrates computer simulation platforms with economic, social and environmental assessment tools in order to evaluate the sustainability of different sugarcane biorefinery systems. In this framework, computer simulation provides technical process data which are used for the sustainability and competitiveness assessment. The assessment is performed considering economic, social and environmental indicators derived from the integration of Economic Engineering, Input-Output Analysis and Life Cycle Assessment. Among other results the VSBI showed the importance of integration of 2nd generation (2G) biorefinery to the sugarcane mills (either autonomous or annexed plants) and helped to identify and quantify some of the main technological bottlenecks of 2nd generation ethanol production. Results also show that ethanol production cost (1G and 2G) is reduced using current 2G production technology in the integrated process, although profitability might be decreased; production of other high added value products in the biorefinery may help to encourage adoption of 2G ethanol production in sugarcane mills, including biogas production through sludge biodigestion and the use of 2G ethanol production residues as fuel in boilers, which allow all bagasse and trash available in the industry to be used as feedstock for 2G ethanol production; agricultural stage has a strong impact on both environmental and economic impacts of ethanol production and improvements on this stage may lead to significant gains. Regulations depending on the materialial impacts are allocated for electricity or lignocellulosic material, environmental gains on the integrated 1G and 2G generation ethanol production may exist. These results show that the proposed framework can be used to design, evaluate and optimize future renewable energy systems with regard to its technical and sustainability aspects.

WE 450
Sustainable material flows in wind energy plants
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Due to the increasing use of wind energy and the recent conscience about taking care of the environment the human being is looking for renewable energies, which allow us to use energy without wasting resources or damage our environment.
Over the five-year period end-2004 to 2009, annual growth rates for cumulative wind power capacity averaged 27 percent. The capacity installed in 2009 is equal to nearly a quarter of total global installations, and cumulative capacity has doubled in less than three years. In the EU, wind power is by far the most popular electricity generating technology. For the second year running wind energy had the largest market share: of almost 26 GW installed in the EU in 2009, wind power accounted for 39%. All renewable technologies combined accounted for 61% of new power generating capacity.
Due to the fact that wind energy is renewable, we want to achieve that most materials needed for windmills shall be recyclable from the beginning until the end. As the tower or other components are made of steel, aluminum or recyclable materials most of the whole windmill is recyclable. If it comes to the recycling of rotor blades which consist mainly of fiber glass combined with other materials there is still no feasible solution to separate all materials and often you need a lot of energy for the processing. Therefore, it is necessary to avoid as much of these materials as possible if it isn't included in the product.
The presentation will give an overview on material flows needed for the wind energy generation and what we might have to deal with in 20 or 30 years. Is wind energy really sustainable? What are we going to do with all the waste of used rotor blades for example?

WE 451
Life cycle management on energy and material resources
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The increasing scarcity of material and fossil resources, the global competition on limited and high valued materials for High Tech products and the coincidence of increasing costs are urgent drivers for improving sustainable production and services. The Social Dimension is challenged through the working conditions of sourcing processes on limited materials or on materials being critical for producing and involving simultaneously the economic and social development and prosperity of transition and developing countries. This will be considered under LCA assessments.
This paper will discuss the use of LCA-Life Cycle Assessment and LCC-Life Cycle Costing for developing ecodesign criteria of Products. The European legislation on 'Energy related Products' requires a Life Cycle Assessment (LCA) -based approach to develop criteria for ecologically sound Products. Re-Use and recycling is of growing importance for limited and high value materials such as noble earths. For this purpose, LCA -based methodology is being used as a management tool for decision-making.
The selected products are characterized by high market volume of appliances, environmental relevance and potentials for improvement.
They comprise among others Consumer Electronics, Household Appliances and Pumps, Lighting etc. being used in private households, industry and public institutions.
LCA methodology includes: goal and scope definition, inventory analysis, impact assessment, interpretation and improvement options/proposals including decision-making process.
Additional Management Systems such as Energy Management follow procedurally the same strategy of a systematic inventory analysis, impact assessment and improvement analysis like LCA. Also recording, monitoring and review are comparable. An important additional component is the active involvement of personnel/workers in supporting the responsible implementation and application of procedures to make the sustainable process operational. This combination of sustainable technology and procedural action and internalization through the personnel confirms the need of the whole integrative process (Life Cycle Sustainability Assessment- LCSA =LCA+LCC+S-LCA).
The next step will be the development of a Resource Efficiency and Resources Management System following the same structure as the other well-experienced Management Systems.

WE 452
Environmental assessment of enhanced geothermal systems through life-cycle assessment and induced seismicity risk criteria
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The development of geothermal systems based on enhanced low-temperature reservoirs is arousing attention as a promising alternative for electricity production using renewable energy.
However, promulgating the development of this technology is inappropriate without a critical assessment of its impacts on the environment. In fact despite the low emissions during the operation phase, large quantities of energy and materials are required for the construction of the plant. The investigation of the impacts of enhanced geothermal systems (EGS) from a cradle to grave perspective can be effectively performed through the life-cycle assessment (LCA) methodology with a relevant set of environmental criteria. Such customized assessment is applied to the analysis of ten significant EGS design options in central Europe. A large variety of independent factors, related both to reservoir conditions and technical choices, determines the size of the installation and the final amount of electricity delivered to the grid. Hence, different sets of parameters are defined to identify the relevant scenarios, accounting the influence of the geothermal flow rate and its temperature, the number and the depth of the boreholes, the reinjection strategy, the power supply process at drilling stage and the risk of induced seismicity.
The latter is a key factor that is progressively gaining importance at design stage, especially after the suspension of the project in Basel (Switzerland) and the experiences of current EGS installations. Hence seismic risk has been identified as a fifth criteria, together with the four end points proposed by the method IMPACT2002+, to assess the environmental impacts of EGS along a complete life cycle approach criteria. The latter is a key factor that is progressively gaining importance at design stage, especially after the suspension of the project in Basel (Switzerland) and the experiences of current EGS installations. Hence seismic risk has been identified as a fifth criteria, together with the four end points proposed by the method IMPACT2002+, to assess the environmental impacts of EGS along a complete life cycle approach criteria.
Results show that impacts of EGS are comparable to those of other renewable energy technologies and significantly lower than those of conventional power plants. The comparison among the ten scenarios enables to formulate recommendations on the environmental suitability of their design. Finally, the risk of induced seismicity is highlighted as a key discriminating factor, as it increases inversely with the environmental benefit.

WE 453
Comparative life cycle assessment of long-lasting insecticidal nets - focus on human health benefits and impact calculation
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Malaria kills more than one million people each year, mostly in sub-Saharan Africa where malaria is the leading cause of death for children under five. Bayer CropScience Environmental Science (BCS-ES) has performed a comprehensive environmental impact assessment of three different mosquito nets using Life Cycle Assessment (LCA) methodology. The LCA was performed with the help of a virtual sugarcane biorefinery framework. Both the eco and Life Cycle Costing (LCC) were used (1) to identify strategies to be explored further for impact reduction and (3) to assess the human health benefits related to mosquito net use and compare them to the adverse potential impacts of net production.
The considered system is referred to the following Functional Unit (FU) “offer an efficient solution of persons against malaria with a mosquito net during a period of time encompassing 50 years”. In the study, the effectiveness of the LLIN is only guaranteed by the persistence of insecticide (based on World Health Organization Pesticide Evaluation Scheme (WHOPE) evaluation).
Using a mosquito net allows the protection from mosquito bites and therefore from malaria and its consequences in terms of morbidity and mortality. Thus, mosquito nets use phase benefits on human health have been assessed, and compared to the potential life cycle human health impacts of the nets. The impact on human health include the contributions from traditional impact pathways such as respiratory effects, toxicity impacts, photochemical ozone formation and from climate change.
The most impacting life cycle stage regarding all the impacts is the mosquito net production. The sensitivity analyses also show that the physical resistance and the end user acceptability are parameters that could strongly influence the overall results.

Under the limitation that calculation of averted DALY's thanks to the mosquito net protection is based on rough assumptions, the results reveal that the benefits of using the nets are about five orders of magnitude in comparison to high IS PS 2006 and in potential impacts generated on human health due to their production, use and disposal, meaning that the benefits of calculating the mosquito nets exceed the environmental burdens.

WE 454
Sustainability Life Cycle Analysis of best available techniques applied to the ceramic industry
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The environmental benefits in terms of monetisation for environmental and health impacts within the framework of Life Cycle Assessment. In case studies they often produce different results. The aim of this paper is to demonstrate on the example of several elementary flows the extent to which the results of the characterisation are influenced by the monetisation method applied. Some underlying differences between the currently available monetisation methods will be discussed as well. Among the methods that are used for this purpose are the CWP 2006 method, the EcoTax method, the ReCiPe method and the EcoSense method.

The results have shown that the characterisation results for a single pollutant or resource (expressed as EUR/tonne) vary considerably depending on the monetisation method applied. For some pollutants the obtained characterisation methods are within a narrow range of values (e.g. CO2) but for others the difference can be up to several orders of magnitude (e.g. Cd and PM10).

Despite their use for the same purposes, the five methods covered by this analysis differ in what they try to achieve, in the effects they consider, in the depth of analysis, the time frame and the choice of the characterisation models and in the way economic values are derived. All these characteristics of the method will determine the calculation procedure and the monetary value which the monetisation method assigns to various emissions and interventions.

WE 455
Comparison of external costs of impacts on human health with and without equity weighting for selected source regions in the Southern Hemisphere
F.E. Kiss
University of Novi Sad, Novi sad, Serbia
The results have shown that the characterisation results for a single pollutant or resource (expressed as EUR/tonne) vary considerably depending on the monetisation method applied. For some pollutants the obtained characterisation methods are within a narrow range of values (e.g. CO2) but for others the difference can be up to several orders of magnitude (e.g. Cd and PM10).

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WE 456
Monetisation of environmental impacts through integrated assessment
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The aim of this work is the development of a methodology for assessing from an environmental, economic and social perspective, different Best Available Techniques (BATs) proposed within the framework of the EU Directive on Industrial Emissions. Taking as a starting point a baseline scenario whose life cycle inventory data have been collected directly from 20 Spanish ceramic tile facilities, thirteen alternative scenarios have been proposed by incorporating to the baseline scenario different BATs related to energy efficiency, dust emissions removal, abatement of the gaseous compounds and noise reduction.

The proposed methodology is based on a combination of methods to assess the effect that each BAT (or combination of BATs) produces on the baseline scenarios: 1) Life Cycle Assessment methodology allows the analysis of the environmental improvement by means of indicators for different impact categories; 2) Eco-efficiency allows to estimate the relationship between economic costs and environmental impacts and 3) Survey to different stakeholders involved in the ceramic sector allows to consider their degree of knowledge and preferences for each BAT.

LC05P - Monetisation for weighting and aggregation in Life Cycle Impact Assessment and Cost-Benefit - Assessment
WE 457
Methods for monetisation in LCA and their impact on the value of characterisation factors
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University of Novi Sad, Novi sad, Serbia

The external environmental cost of residential buildings in Belgium was estimated during a four year project - SuFiQuaD (Sustainability, Financial and Quality evaluation of residential buildings).

Integrating life cycle environmental costs in decision making, applied to Belgian residential buildings
V. Bátiz Zúñez, M.D. Bovea, A. Azapagic
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2University of Novi Sad, Novi Sad, Serbia

The environmental benefits in terms of monetisation for environmental and health impacts within the framework of Life Cycle Assessment. In case studies they often produce different results. The aim of this paper is to demonstrate on the example of several elementary flows the extent to which the results of the characterisations are influenced by the monetisation method applied. Some underlying differences between the currently available monetisation methods will be discussed as well. Among the methods that are used for this purpose are the CWP 2006 method, the EcoTax method, the ReCiPe method and the EcoSense method.

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Despite their use for the same purposes, the five methods covered by this analysis differ in what they try to achieve, in the effects they consider, in the depth of analysis, the time frame and the choice of the characterisation models and in the way economic values are derived. All these characteristics of the method will determine the calculation procedure and the monetary value which the monetisation method assigns to various emissions and interventions.

WE 458
Aggregation of multiple environmental benefits
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There are currently several methods in use for monetisation of environmental and health impacts within the framework of Life Cycle Assessment. In case studies they often produce different results. The aim of this paper is to demonstrate on the example of several elementary flows the extent to which the results of the characterisations are influenced by the monetisation method applied. Some underlying differences between the currently available monetisation methods will be discussed as well. Among the methods that are used for this purpose are the CWP 2006 method, the EcoTax method, the ReCiPe method and the EcoSense method.

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Despite their use for the same purposes, the five methods covered by this analysis differ in what they try to achieve, in the effects they consider, in the depth of analysis, the time frame and the choice of the characterisation models and in the way economic values are derived. All these characteristics of the method will determine the calculation procedure and the monetary value which the monetisation method assigns to various emissions and interventions.

WE 459
Integrating life cycle environmental costs in decision making, applied to residential buildings
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The external environmental cost of residential buildings in Belgium was estimated during a four year project - SuFiQuaD (Sustainability, Financial and Quality evaluation of Dwelling types). The aim was to formulate recommendations to move towards a more sustainable building stock. It was investigated if current decisions based on financial consideration would lead to a more sustainable housing stock. Policy recommendations were formulated by identifying the measures in order of priority to reduce the environmental impact of our building stock and by investigating if internalisation of the external environmental cost could enhance sustainable building.

The research methodology consisted of several steps. In a first step existing methods of monetization were compared. Based on this comparison, a hybrid approach was used combining the Clean for Air Europe (CAFE) method with several other literature sources. The methodology proposed was then implemented - in combination with a life cycle costing analysis - in three typical case studies: optimisation of building elements, optimisation of 16 representative dwellings and evaluation of current policy incentives (subsidies and tax reduction) related to sustainable building.

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In conclusion, comparing the results obtained through €CO model with the methods above described, it is possible to affirm that the scale of values is substantially unaltered.

Model which takes into account individual heterogeneity- provides marginal WTPs for each impact category.

(CE) is a stated preferences method which requires to design a survey and then to submit it to a population sample (a test sample of one hundred persons). People are asked to:

- assign weights to the different pollution categories (three: air, water, and land);
- provide a monetary value for the reduction of pollution that is equal to the one they expect to obtain thanks to the new technology.

A variety methods are risen in the two last decades in order to include the social and environmental costs and benefits into economic evaluations.

In this study, a new assessment model is proposed to investigate the impacts from resources use, as none of the existing methods is considered mature enough to be used in practice. Several experimental tests were performed and the results showed the reliability of the new approach. The new approach is based on a life cycle costing (LCC) methodology and includes environmental impacts.

The aim of this paper is to show the results connected to a study for a new assessment model based on the integration of LCA and LCC, called €CO (Politecnico di Torino, Thebaet, 2009). Different methods, get from published international researches, have been evaluated to apply the same building envelopes in order to validate and verify the outcome of €CO model. Methodologies represented and implemented are based either on the monetization of externalities, as the European ExternE Project and the Swedish EPS method, or on scientific studies that take into account environmental and health impacts without weighting them, such as SETAC Environmental LCC and CES Selector, the methodology developed by Prof Mike Ashby (Cambridge University).

Monetary further efforts to validate the results is the application of the Emission Trading (EU Dir. 2003/87/EC), market-based approach used to control pollution by providing economic incentives for achieving reductions in the emissions of pollutants. In other words, the research tries to shift this approach to the construction market applying the Marginal Abatement Cost Curve (MAC), that is the cost of eliminating an additional unit of pollution.

In conclusion, comparing the results obtained through €CO model with the methods above described, it is possible to affirm that the scale of values is substantially unaltered.

Monetization of indirect externalities of lead emissions from a waste-to-energy plant with focus on trade-offs related to the time horizon choice

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In this study we calculated, via the impact pathway approach (IPA), the indirect external costs of lead emitted from a waste-to-energy facility in Denmark. After emission into the atmosphere, lead deposited on topsoil can be uptaken via ingestion. Lead exposure is linked to neurotoxic impacts in children and to reduced lifetime earnings. Indirect costs are expected to be higher than direct ones (via inhalation): lead accumulates in topsoil and ingestion is a major contributor to total daily lead intake. However, indirect impacts occur in the future; their present value is therefore lower when a positive discount rate is applied. Calculating the indirect externalities poses some challenges: a) need for the additional air-to-soil dispersion modelling; b) need for a long-term perspective to account for lead accumulation in soil; c) impacts and costs to be integrated over future generations. The choice of the time horizon can potentially influence the final results in terms of total costs [€/kg]. We used different models cross-disciplinarily inside the IPA framework: the Operational Meteorological air-quality model (OML), the Simplified Fate and Speciation Model (SFSM), and the Age Dependent Biokinetic Model (ADBM) were used to determine respectively the metals' atmospheric transport, its deposition and accumulation in topsoil, and its bio-accumulation in the human body. Neurotoxic impacts were quantified according to different long-term emission and exposure scenarios; concentration-response functions from updated literature were used, linking blood lead and IQ. The economic model to calculate lifetime earnings has been adapted to monetise neurotoxic impacts on future generations of children. Calculated indirect costs are of the same magnitude as direct costs (previously investigated), both in the case of actual emission rates and in the case of future emission scenarios for lead. Among the various modelling parameters, the social discount rate has the most influential effect: discounting of future impacts is levelling off the differences between indirect and direct costs, so that even if lead is accumulating in soil with a consequent increasing exposure, the present monetary value of future impacts doesn’t increase. The use of lead-specific models allows for a detailed quantification of the lead impact pathway and results are in agreement with previous literature.

Monetisation of environmental impacts from LCA with the Choice Experiment method: an experimental approach for waste management systems

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Life Cycle Assessment (LCA) in its most advanced version has been the most used method for environmental planning at a local or regional scale. This tool allows comparison of scenarios according to their different impacts. The implementation of a monetisation method on these results can be seen as a possibility to establish a hierarchy of waste management scenarios to help decision-making.

Contrary to classical monetisation methods, which are mainly based on damage or on emissions costs, we propose to monetise environmental impacts from a LCA at a mid-point level. The originality of the method relies on the implementation of a monetisation method on these results can be seen as a possibility to establish a hierarchy of waste management scenarios to help decision-making.

Our explanatory approach begins with a LCA implementation by modelling with GaBi software four waste management scenarios: a benchmark scenario (incineration) and three Mechanical Biological Treatment alternatives. Waste management systems are limited to residual waste and biowaste flows and take into account collection, transport, treatment, refusal management and by-products valorisation. Only three midpoint impact categories, which represent issues in this area, are studied: Global Warming Potential (GWP), Acidification Potential (AP) and Bioaccumulation Potential (BP).

Then, we propose to adapt the Choice Experiment method, derived from marketing research, to obtain monetary values from environmental impacts. Choice Experiment (CE) is a stated preferences method which requires to design a survey and then to submit it to a population sample (a test sample of one hundred persons). People are asked to consider different scenarios based on the different values they have to directly express their Willingness-To-Pay (WTP).

An econometric analysis of the survey results - here a Mixed Logit Model which takes into account individual heterogeneity - provides marginal WTPs for each impact category.

CE is generally applied to real issues. That’s why we need to adapt it by bringing additional information about the topic on the one hand and by giving a “picture described of environmental impacts on the other hand. Results stress the existence of an important intra impact dispersion of individual WTPs and important inter impact differences in average WTPs.

WEPC1 - Bioavailability and bioaccumulation - impact of environmental, biological and ecological variation

WEPC1-1
Uncertainty of bioavailability and biomagnification measurements in natural aquatic food webs: a sensitivity analysis approach

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The potential of a chemical substance to accumulate in aquatic organisms and to increase in concentration with increasing trophic level are criteria that are used to classify chemicals as bioaccumulative or bioconcentrative. For these reasons, it is important to determine the bioaccumulation of contaminants by living organisms relative to the amount stored in the water compartment. Similarly, biota-sediment accumulation factors (BSAFs) describe the accumulation of contaminants by living organisms relative to the amount stored in the sediment compartment. Bioaccumulation factors (BMFs) describe the increase in concentration of a substance in living organisms relative to a single trophic level step on a food chain. Because of biomagnification processes, individuals BAF or BSAF values are dependent upon trophic level position of the organism under consideration. Consequently, trophic magnification factors (TMFs), which describe the increase in concentration of a substance in living organisms that occupy successively higher trophic levels within a food web, are used to assess bioaccumulation and biomagnification of chemicals in the environment. This presentation will show how the methods for assessment of bioavailability and biomagnification (i.e., BAF, BSAF, BMF, and TMF) are interrelated. We will use field level data to evaluate various approaches that may be used to calculate bioaccumulation and biomagnification metrics from measured concentrations, normalized concentrations...
Most bioaccumulation models assume that uptake occurs via passive diffusion through a series of water and lipid layers. However, organisms have developed active transport in the PBT assessment. Various examples have shown that metabolites of chemicals are more toxic than the parent compounds. B-assessment should therefore also include biotransformation as a key factor in determining bioaccumulation and further trophic magnification. In the PBT assessment, much attention is being paid to the persistence factor, which can be used to estimate changes in element translocation in plants. Change of element participation in metabolism can be expressed as dynamic factors of biophilicity.

The proposed revision to the OECD 305 Test Guideline, Bioconcentration: Flow-through Fish Test, includes an approach for exposure via the diet for substances that are not bioconcentrable. Comparison of fish species used for dietary exposure according to the proposed OECD 305 test guideline may be made. Environmental, biological, and ecosystem variables may affect the transfer of contaminants from the abiotic to the biotic compartment, including variation in contaminant concentration and flux, as well as in physiological or biotransformation ability. Since element participation in metabolism is affected by the physiological or biotransformation ability on the total concentration of soil we use a new biogeochemical - dynamic bioaccumulation factor - to analyze change in bioaccumulation. Dynamic translocation factor is used to estimate changes of element translocation in plant. Change of element participation in metabolism can be expressed in dynamic factor of biophilicity. Dynamic factors are different from the factors mentioned in the existing literature, highlight changes in processes rather than changes in element quantities when conditions of the environment change. Equations to calculate factors as well as a case study will be presented and discussed in the presentation.

Comparison of fish species used for dietary exposure according to the proposed OECD 305 Test Guideline A. Lillicrap, K. Baek, K.A.T.H.E. Langford
SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting

The proposed revision to the OECD 305 Test Guideline, Bioconcentration: Flow-through Fish Test, includes an approach for exposure via the diet for substances that are highly hydrophobic and not suitable for aqueous exposure. To validate the dietary exposure method, an international ring test was performed to determine the interlaboratory variability of the proposed test method. In the ring test, different US estuaries, the Chesapeake Bay and San Francisco Bay, was compared to model predictions. A biokinetic model combining aqueous and dietary uptake terms was modified such that the dietary uptake term might pose an additional uptake pathway for environmental pollutants and whether it should be included into risk assessment.

Metal bioaccumulation in deposit-feeding polychaete depends on sediment geochemistry Z. Baumann, N.S. Fisher
Stony Brook University, Stony brook, United States of America

Current understanding of metal bioaccumulation in deposit-feeding animals is still unresolved. Field observations need to be complemented with laboratory-based studies and mathematical modeling of metal uptake. Polychaetes collected from different US estuaries, the Chesapeake Bay and San Francisco Bay, was compared to model predictions. A biokinetic model combining aqueous and dietary uptake terms was modified such that the dietary uptake term was considered parameterizing of the metals in diverse geochemical fractions as determined with a sequential extraction scheme. Kinetic parameters, including metal assimilation efficiencies and ef flux rate constants, that were used in the biokinetic model to parameterize data from the Chesapeake Bay and San Francisco Bay, was compared to model predictions. A biokinetic model combining aqueous and dietary uptake terms was modified such that the dietary uptake term might pose an additional uptake pathway for environmental pollutants and whether it should be included into risk assessment.

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First results of the 2DE are showing that both drugs have the ability to induce a specific response in blue mussels after 14 days of exposure. To validate the dietary exposure method, ring testing has been undertaken to demonstrate reproducibility of results, and provide information on interlaboratory variation. Ten laboratories worldwide conducted dietary bioaccumulation studies, using rainbow trout. Five test substances were chosen to represent a range of potential for biomagnification: hexachlorobenzene, musk xylene, ortho-terphenyl, methoxychlor, benz[a]pyrene. The substances were spiked to commercial fish food, on which fish were fed daily during a set exposure regime at two different feed rates, followed by depuration on substance-free food. Chemical analysis of fish tissue during depuration, along with basic substance and organism data, allows for calculation of a fish growth rate constant, a substance depuration rate constant, assimilation efficiency, and biomagnification factor. Results from the ring test are described, including information on feeding rate, corrections for fish growth during the test, reproducibility and interlaboratory variation.

**WEPC2** - Environmental OMICs: a global answer to environmental questions

**WEPC2-1**
Evaluation of chronic sublethal effects of the pharmaceuticals gemfibrozil and diclofenac on the marine mussel (Mytilus spp.) using a proteomic approach

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Since the 1980s, the stocks of European eel have been declining in most of their geographical distribution area and they are now considered below safe limits for population maintenance. Factors that have contributed to this decline include habitat loss and degradation, fishing, pollution, environmental stressors and viral disease. The marine mussel (Mytilus spp.) was exposed to environmentally relevant and elevated concentrations (1 & 1000 µg/L, respectively) for 14 days. Chronic sublethal effects will be investigated by looking at the protein expression signatures using two-dimensional gel electrophoresis (2DE) and a suite of biomarkers comprising glutathione transferase, lipid peroxidation and DNA damage. Additionally it will be examined whether blue mussels have the potential to recover from an exposure. Therefore mussels were left to recover for an additional seven days after the treatment. First results of the 2DE are showing that both drugs have the ability to induce a specific response in blue mussels after 14 days of exposure. This study contributes to the understanding of the biological effects of pharmaceuticals in non-target organisms, such as the widespread blue mussels.

**WEPC2-2**
Protein expression profiles in European eel (Anguilla anguilla) exposed to perfluorooctane sulfonate; combination of in vitro, in vivo and in situ approaches

K.R. Roland1, P. Kestemont2, M.A. Pierard3, M. Raeu1, M. Dieu1, R. Loos2, B. Gawlik2, F. Silvestre2
1UNamur FUNDP, Namur, Belgium 2University of Namur FUNDP, Namur, Belgium 3University of Namur FUNDP, Namur, Belgium

Since the 1990s, the stocks of European eel have been declining in most of their geographical distribution area and they are now considered below safe limits for population maintenance. Factors that have contributed to this decline include habitat loss and degradation, fishing, pollution, environmental stressors and viral disease. The marine mussel (Mytilus spp.) was exposed to environmentally relevant and elevated concentrations (1 & 1000 µg/L, respectively) for 14 days. Chronic sublethal effects will be investigated by looking at the protein expression signatures using two-dimensional gel electrophoresis (2DE) and a suite of biomarkers comprising glutathione transferase, lipid peroxidation and DNA damage. Additionally it will be examined whether blue mussels have the potential to recover from an exposure. Therefore mussels were left to recover for an additional seven days after the treatment. First results of the 2DE are showing that both drugs have the ability to induce a specific response in blue mussels after 14 days of exposure. This study contributes to the understanding of the biological effects of pharmaceuticals in non-target organisms, such as the widespread blue mussels.

**WEPC2-3**
Response of the European flounder Platichthys flesus to experimental dietary exposure and in situ contaminations: a proteomic approach

C. Galland1, C. Dupuy2, I. Calve1, V. Loizeau1, L. Quiniou1, M. Dieu1, R. Loos1, B. Gawlik2, F. Silvestre2
1UNamur FUNDP, Namur, Belgium 2University of Namur FUNDP, Namur, Belgium

In the early 1990s, the stocks of European eel have been declining in most of their geographical distribution area and they are now considered below safe limits for population maintenance. Factors that have contributed to this decline include habitat loss and degradation, fishing, pollution, environmental stressors and viral disease. The marine mussel (Mytilus spp.) was exposed to environmentally relevant and elevated concentrations (1 & 1000 µg/L, respectively) for 14 days. Chronic sublethal effects will be investigated by looking at the protein expression signatures using two-dimensional gel electrophoresis (2DE) and a suite of biomarkers comprising glutathione transferase, lipid peroxidation and DNA damage. Additionally it will be examined whether blue mussels have the potential to recover from an exposure. Therefore mussels were left to recover for an additional seven days after the treatment. First results of the 2DE are showing that both drugs have the ability to induce a specific response in blue mussels after 14 days of exposure. This study contributes to the understanding of the biological effects of pharmaceuticals in non-target organisms, such as the widespread blue mussels.

**WEPC2-4**
Constructing estrogen-sensitive gene networks in the liver of Fundulus heteroclitus for characterizing adverse effects of estrogenic pollutants

1University of British Columbia, vancouver, Canada 2University of Stirling, Stirling, United Kingdom 3University of Stirling, Stirling, United Kingdom 4University of Stirling, Stirling, United Kingdom 5University College Cork, Cork, Ireland 6University of Brest-IUEM-LEMAR, Plouzané, France 7University of Brest-IUEM-LEMAR, Plouzané, France 8University of Brest-IUEM-LEMAR, Plouzané, France

In this study, the marine mussel (Mytilus spp.) was exposed to environmentally relevant and elevated concentrations (1 & 1000 µg/L, respectively) for 14 days. Chronic sublethal effects will be investigated by looking at the protein expression signatures using two-dimensional gel electrophoresis (2DE) and a suite of biomarkers comprising glutathione transferase, lipid peroxidation and DNA damage. Additionally it will be examined whether blue mussels have the potential to recover from an exposure. Therefore mussels were left to recover for an additional seven days after the treatment. First results of the 2DE are showing that both drugs have the ability to induce a specific response in blue mussels after 14 days of exposure. This study contributes to the understanding of the biological effects of pharmaceuticals in non-target organisms, such as the widespread blue mussels.

**WEPC2-5**
A ring test of the draft OECD 305 bioconcentration test guideline dietary exposure method

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1UNamur FUNDP, Namur, Belgium 2University of Namur FUNDP, Namur, Belgium

In the early 1990s, the stocks of European eel have been declining in most of their geographical distribution area and they are now considered below safe limits for population maintenance. Factors that have contributed to this decline include habitat loss and degradation, fishing, pollution, environmental stressors and viral disease. The marine mussel (Mytilus spp.) was exposed to environmentally relevant and elevated concentrations (1 & 1000 µg/L, respectively) for 14 days. Chronic sublethal effects will be investigated by looking at the protein expression signatures using two-dimensional gel electrophoresis (2DE) and a suite of biomarkers comprising glutathione transferase, lipid peroxidation and DNA damage. Additionally it will be examined whether blue mussels have the potential to recover from an exposure. Therefore mussels were left to recover for an additional seven days after the treatment. First results of the 2DE are showing that both drugs have the ability to induce a specific response in blue mussels after 14 days of exposure. This study contributes to the understanding of the biological effects of pharmaceuticals in non-target organisms, such as the widespread blue mussels.
also include gene expression analysis on developing larvae from 50 individual larvae. Gene expression oligonucleotide microarray is an important tool in the search for new biomarkers and understanding the molecular mechanism behind physiological changes. We will here present results from a much larger gene expression study including 16 sites around the Baltic Sea area involving 160 individual eelpout samples. Results will also include gene expression analysis on developing larvae from 50 individual larvae. Gene expression oligonucleotide microarray is an important tool in the search for new biomarkers and understanding the molecular mechanism behind physiological changes. We have earlier sequenced the eelpout liver transcriptome and the sequence data has been used to design and construct an oligonucleotide microarray. This eelpout microarray study identifies novel gene networks for cell pathways underlying the mechanisms of action of EE2 for egg production. We used shotgun and suppression subtraction hybridization (SSH) cDNA libraries followed by 454 FLX sequencing (957,303 reads sequenced in total) and RT-qPCR to study the effects of POPs in eelpout from Lake Mjøsa. The gene list of putatively higher or lower expressed genes in liver of burbot from Lake Losna compared to liver of burbot from Lake Mjøsa, generated from the SSH cDNA libraries, suggest that mechanisms associated with cancer, lipid metabolism and vitamin and mineral metabolism are differentially regulated in the two populations. Ingenuity Pathway Analysis (IPA) top networks according to the IPA analyses, the top toxicity list ranking was: “Negative/Positive acute phase response proteins”, “FXR/RXR activation”, “NRF2-mediated oxidative stress response” and “Glutathione depletion - CYP induction and reactive metabolites”. Overall, the study shows that next-generation sequencing may be a valuable supplement or alternative to microarray technology in toxicogenomic discovery of environmental samples.

That of environmental samples. Moreover, it shows that it is possible to expand high-capacity toxicity testing on periphyton communities to include also effects on the genetic structure or metagenomic composition of communities. The toxic effect of these compounds on pigment profiles were compared to their effect on genetic structure of the communities, using Temperature Gradient Gel Electrophoresis (TGGE) methodology. The communities in the SWIFT tests are also compared to communities sampled at several locations in the Baltic Sea area. The toxic effect of these compounds on pigment profiles were compared to their effect on genetic structure of the communities, using Temperature Gradient Gel Electrophoresis (TGGE) methodology. The communities in the SWIFT tests are also compared to communities sampled at several locations in the Baltic Sea area.

The impact of pesticides on the benthic macroinvertebrates in a rural lake
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Human activities at the landscape scale are a threat to the ecological integrity of many aquatic ecosystems. It is often difficult to isolate the effects of pollutants from other causative factors, and to identify the primary pollutants causing ecosystem stress. Additionally, pollutants may not be homogenously dispersed, with potentially varying effects on biota living within the same water body; therefore there is a need to have appropriate strategies for management in order to maintain the ecological integrity of the whole ecosystem.
Ecosystem. The study was undertaken at Lake Hawthorn in northwestern Victoria, Australia. Results from previous surveys of the lake showed that sediments were impacted by a number of pesticides which were also toxic to macroinvertebrates. Pesticides were not dispersed homogeneously throughout the lake. The aims of the study were to a) determine the number of samples that should be collected to ensure that there is an adequate assessment of contaminants present in sediment and water and b) determine if the persistence and health of benthic macroinvertebrates are affected by the presence of pesticides. The lake was divided into six sampling locations and between three and five sites selected at each location, where sediment and surface water was collected and a range of chemicals were measured (including metals, a range of pesticides and nutrients). Benthic macroinvertebrates were sampled in a subset of sites at each location for species abundance and diversity and also for changes at the sub-organism level on a suite of biochemical biomarkers. Sediments were also assessed for toxic effects on survival, growth and emergence of Chromatium tepperi using a laboratory-based bioassay and impacts on indigenous macroinvertebrates using field-based microcosms. There were elevated concentrations of copper and total petroleum hydrocarbons in sediment at one location within the lake, an urban inlet drain. There were also effects on C. tepperi survival at this location. Furthermore, there were no in situ chironomids collected. Subsequent biomarker analyses on the chironomid Tanytarsus semibarbataeus collected in situ showed that chironomids are responding differently within the lake. Our results show that the pollutants present in Lake Hawthorn vary spatially and that they are toxic to aquatic life. Results on sampling strategically and the usefulness of a multiple lines of evidence approach for aquatic biomonitoring will be discussed.

WEPC3-2
Understanding the legacy of aged p,p'-DDT and p,p'-DDE residues in New Zealand horticultural soils

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The legacy of p,p'-DDT and p,p'-DDE residues in New Zealand horticultural soils is discussed. Conclusions of a multiple lines of evidence approach for aquatic biomonitoring will be discussed.

WEPC3-3
Impacts of sediment-bound synthetic pyrethroids on non-target aquatic macroinvertebrates

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What is persistence? Seeking for a practical interpretation for regulatory practice

WEPC4 - Tropical ecotoxicology

WEPC4-1 Combining natural competitors and larvaeicides to improve mosquito control in Cameroon and Kenya

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Recently a tremendous number of infections and deaths in Africa are caused by malaria and other mosquito-borne diseases. Since 2005 the potential of using larvaeicides and natural competitors to reduce mosquito larvae, as well as the amount of chemicals applied has been successfully demonstrated in the floodplains of river Elbe in Germany and in outdoor ponds in Cameroon. To test the feasibility of this strategy on a larger scale, extended field trials are currently conducted in different habitat types and regions (Nyabondo plateau in western Kenya and the surrounding area of Yaoundé in Cameroon). Preliminary results indicate the success of the use of natural competitors and larvaeicides dependent on the mosquito species targeted and the density of mosquitos present in the aquatic habitats. Combining traditional measures and those suggested by the novel approach promises to enhance the capability of African communities and their national programs to improve the sustainable control of malaria and other mosquito-borne diseases.

WEPC4-2 Fate of polychlorinated biphenyls (PCBs) in the surface soil of a subtropical forest

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Forest (TBR) is a biocide that has been widely used in anti-fouling paints for ships. In order to assess the status of TBT pollution in Walr Harbours, imposex was studied in the Nigerian periwinkle, Tympanotonus fuscatus var. radula an important shellfish in the Niger Delta. The survey results showed that TBT concentration and imposex development were generally low in areas with high intensity of boat and vessel traffic. T. fuscatus var radula was then exposed to sublethal concentrations (0.1, 1.0, 10 and 70μg/L) for 21 days. The effect on test organisms was monitored for 10 days through imposex expression (grades 0-4) and in the Niger Delta ecological zone.

WEPC4-3 Weed invasion at the rehabilitated Mary Kathleen uranium mine site, northern Australia: toxicity risk to grazing animals

G. Lottermoser

University of Tasmania, Hobart, Australia

Calotropis procera (also known as Sodom apple) is a xerophytic shrub or small tree species, which is native to tropical and subtropical Africa, Asia and the Middle East. C. procera is now naturalised in Australia, Central and South America, the Caribbean islands, Indonesia, and many Pacific islands. The plant is alleged to be toxic to humans, cattle, sheep, rabbits and mice. To assess the impact of this study was conducted at the rehabilitated Mary Kathleen uranium mine site, northern Australia. Since rehabilitation in the 1980s, C. procera has colonized all rehabilitated mine site domains and the plant now represents a widespread and abundant pest. Biochemical analyses indicate that C. procera acquires natural enrichments of Ca, Mg and S in its tissue on mine as well as background soils. The concentrations of these elements exceed their maximum tolerable concentrations in the diet of cattle. Considering the chemical toxicity threat to grazing animals due to the presence of steroidal heart poisons and the elevated Ca, Mg and S contents in the plant’s biomass, control of the weed will be required using appropriate management techniques. At Mary Kathleen, neglect of rehabilitated mined lands by statutory authorities has led to the development of unsustainable vegetation covers over disturbed ground and mine waste repositories in the long term.

WEPC4-4 Effect of Tributyltin (TBT) on testosterone metabolism and imposex expression in Tympanotonus fuscatus var radula in Warri Harbour, Nigeria

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The Nigerian periwinkle, Tympanotonus fuscatus var. radula an important shellfish in the Niger Delta. The survey results showed that TBT concentration and imposex development were generally low in areas with high intensity of boat and vessel traffic. T. fuscatus var radula was then exposed to sublethal concentrations (0.1, 1.0, 10 and 70μg/L) for 21 days. The effect on test organisms was monitored for 10 days through imposex expression (grades 0-4) and biomass production in CR and UnC after runoff addition, with a reduction in dissolved oxygen after 3 h. In relation to phytoplankton, density reduction was observed in C (65%), UnC (90.6%) and CR (92.0%), but less effect was observed in V (37.7%). The diversity was smaller in V (0.7 bits/ind) in relation to C, UnC & CR treatments (above 1.7bits/ind) after T240h. Chlorophyceae were more representative in V (45.2%) compared to Y. (7.4%) and besides Baccillariophyceae (C in CR (40%). The chlorophyll concentrations were similar in T (below 10 μg/L) but highest in C (32 μg/L) and UnC (1μg/L) in T240h, with considerable differences in phycocyanin concentrations (C<UnC<V<CR). Rotifiers was important in T0 and T240h (64%). Density reduction (from 58 to 100%) was observed in Protozoa (C,V), Rotifera (C, UnC, RC, V) and Copepoda (RC, V); nevertheless, the results weren’t the same in relation to biomass (C>UnC>RC>1.2 and 2.9 biof/ind, respectively), and the major contribution was related to Baccillariophyceae in C, UnC, CR and V. Also, some differences in numbers and dominant species were observed between the treatments (C<UnC<V<CR). The results indicate that VtB218EC and the runoff were responsible for the modification in the communities’ structure. Besides, direct and indirect effects were observed, which are associated with biological processes (competition, predation and grazing) and abiotic alterations.

WEPC4-5 Direct and indirect effects of pesticides on aquatic communities: an experimental bioassay in tropical systems (São Paulo, Brazil)

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In recent decades there has been an expansion of agriculture to support the increase in demand for food in Brazil. On the other hand, pesticides are being used for farmers and as a result they have been changed in biological activity and functional processes in terrestrial ecosystems. However, pesticides may also directly or indirectly affect the aquatic ecosystems, producing undesirable effects on their biological and functional properties. To evaluate these effects, experimental bioassays were conducted with Verrimic®18EC during 10 days, in different treatments (Control: C; direct application of Verrimic: V; uncontaminated runoff: UnC and contaminated runoff: CR). The results showed that the effect on test organisms was monitored for 10 days through imposex expression (grades 0-4) and biomass production in CR and UnC after runoff addition, with a reduction in dissolved oxygen after 3 h. In relation to phytoplankton, density reduction was observed in C (65%), UnC (90.6%) and CR (92.0%), but less effect was observed in V (37.7%). The diversity was smaller in V (0.7 bits/ind) in relation to C, UnC & CR treatments (above 1.7bits/ind) after T240h. Chlorophyceae were more representative in V (45.2%) compared to Y. (7.4%) and besides Baccillariophyceae (C in CR (40%). The chlorophyll concentrations were similar in T (below 10 μg/L) but highest in C (32 μg/L) and UnC (1μg/L) in T240h, with considerable differences in phycocyanin concentrations (C<UnC<V<CR). Rotifiers was important in T0 and T240h (64%). Density reduction (from 58 to 100%) was observed in Protozoa (C,V), Rotifera (C, UnC, RC, V) and Copepoda (RC, V); nevertheless, the results weren’t the same in relation to biomass (C>UnC>RC>1.2 and 2.9 biof/ind, respectively), and the major contribution was related to Baccillariophyceae in C, UnC, CR and V. Also, some differences in numbers and dominant species were observed between the treatments (C<UnC<V<CR). The results indicate that VtB218EC and the runoff were responsible for the modification in the communities’ structure. Besides, direct and indirect effects were observed, which are associated with biological processes (competition, predation and grazing) and abiotic alterations.

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The ecological effects of agroindustrial farming in Southern Amazon L. Soto1, A. Pigozzo2, C. Civen, Venezia, Italy
1Technical University of Lisbon, Lisbon, Portugal
2Kunsan National University, Kunsan, South-Korea (Rep)

Photocatalytic effects of TiO2 nanoparticles - how do they impact the ecotoxicity of PAHs? A model study with Caenorhabditis elegans J.S. Angelstorf

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Environmentals characterisation, fate, detection, exposure, effect and risk of nanoparticles WECPC5

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products has experienced a massive increase in recent years. For example, sunscreens employing nanosized titanium dioxide (nTiO2) and zinc oxide (nZnO) as UV-blocking agents are becoming more popular as they are deemed to have enhanced effectiveness and transparency. NMs may be released into the aquatic environment during the product life-cycle, but their ecological impacts on marine ecosystems are presently largely unknown. Therefore, this study primarily aimed to investigate the ecotoxicological effects of nZnO on different marine organisms, including the freshwater crustacean Daphnia magna, the marine copepod Tigripus japonicus, and the marine mussels Mytilus edulis. The effects of nZnO were investigated under both static and dynamic exposure conditions to understand the detoxifying effects of nZnO and to evaluate the potential long-term effects on these species.

WEPC3-5
Do TiO2 nanoparticles alter new metal toxicity? - A factorial approach using Daphnia magna
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The present knowledge about the risk of carbon nanoparticles (CNMs) requires the developing of new testing methodologies and interpretative tools to gain a more comprehensive picture of their toxic effects. Aquatic ecosystems are considered the terminal receptors of nanomaterials and their realistic accumulation sites. Lethal and cytotoxic effects are usually assessed by standardized tests and are determined by advanced confocal and electron microscopy techniques. Morphometric CNP properties were characterized by scanning and transmission electron microscopy, and their hydrodynamic behaviour monitored by dynamic light scattering techniques. PAHs content was evaluated by Soxhlet extraction for 48h using toluene. Embryos were acutely exposed to increasing CNP concentrations (1, 10, 100 and 500mg/L) and after 96h, lethality, malformation rate and growth inhibition were measured. Embryos were then processed for histological and ultrastructural analyses to detect the main affected organs, and to look for specific lesions at the subcellular level. Laser Scanning Microscopy in the reflection mode and EFTEM techniques were used to detect CNP accumulation sites. Only the highest CNP suspension resulted embryolethal for X. laevis larvae, while a dose-response was observed for juveniles, uninformed as 3.1% of control larvae, of the 100 and 500mg/L As as well as Cu was reduced from cont-35% to 0.8% and <0.001%, respectively. These results suggest TiO2 as a suitable measure to reduce metal toxicity in distinct cases (e.g. Cu). However, as the underlying processes are not yet fully understood and the detoxifying effects were not apparent for all investigated elements, the application of nTiO2 should carefully be evaluated in future experiments.

WEPC3-6
Nano-silver ecotoxicological effects in marine mussels
F. Dondero, L.E.N. Saggese
Università degli Studi di Milano, Milan, Italy

Effect of Carbon nanoparticles on Xenopus laevis development
N. Santo1, U. Fascio2, C. di Benedetto1, N. Guazzoni1, P. Tremolada1, R. Bacchetta1, C. Camatini1, P. Mantecce1
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2Università di Milano-Bicocca, Milan, Italy

In a study employing five marine organisms (i.e., microalgae Thalassiosira pseudonana and Skeletonema costatum, copepod Tigriopus japonicus, amphipod Eurydice rapax, and medaka Oryzias melastigma), crustaceans exhibited the highest sensitivity towards nZnO, and toxicity of nZnO was attributed to metal ion dissolution as well as nanoparticulate effects such as aggregation and adsorption at nZnO onto cell walls and exoskeletons. Dissolution of nZnO decreased from 16 mg Zn L-1 at 1 ºC to 1.4 mg Zn L-1 at 35 ºC, which probably accounted for the increased growth inhibition for S. costatum at 15 ºC when compared against 25 ºC. However, physiology and metabolism of these freshwater organisms, such as metabolic depression and dormancy displayed by the amphipod Melita longicidula at lower temperatures, could also influence nZnO uptake and toxicity. T. japonicus was exposed to nZnO-incorporated sunscreens to evaluate the effects of commercial forms of nZnO and N2+ leached into seawater surfaces. Their molecular biomarker responses showed that other sunscreen constituents can alter nZnO toxicity, and such results support the need for further research on combined effects of NMs and other common pollutants to aquatic organisms.

WEPC4-2
Can time-weighted average concentrations be used to assess the risks of asulam to Myriophyllum spicatum and Elodea canadensis under different time-variable exposure regimes?
J.D.M. Belgers, M.C. Boerwinkel, L.L. Buijsse-Bogdan, T.C.M. Brock
Alterra, Wageningen, Nederland

We tested the effects of the herbicide asulam on growth of the submerged macrophytes Myriophyllum spicatum and Elodea canadensis under laboratory conditions using different exposure scenarios. Asulam is a selective post-emergent systemic carbamate herbicide which inhibits folate synthesis by inhibiting dihydropteroate synthase. The exposures of each scenario were comparable in the concentration x time factor, viz., the same 21-d time-weighted average (TWA) concentrations but variable in peak exposure concentrations (ranging from 0.1 to 2100 µg ai/L) and exposure periods (1, 3, 7, 14 or 21 days). Endpoints, dry-weight of main shoots, new shoots and new roots and lengths of new shoots and roots were measured at the end of the exposure period (t = 21 days). It appeared that different exposure regimes resulted in similar EC50 values when exposure scenarios were similar (EC50: 59-80 µg/L).

WEPC4-6
Linking exposure to effects in environmental risk assessment

WEPC5-1
Effect modelling of intermittent discharge of herbicides in watercourses
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Ecotoxicology, effects of agricultural nanomaterials on non-target organisms, like algae, are sometimes referred to a continuous exposure. But this kind of exposure is very far from the reality. Indeed, exposure models of pesticides in aquatic systems after agricultural applications and after rain events are generally irregular i.e. characterized by short pulses exposure and recovery periods. Even if they are short, they can impair the growth of algae or modify their chlorophyll content. Very little is known about the effects on primary producers of short but high peaks of herbicides, on how the algae respond during the recovery period between pulses. The main goal of this study is to develop a model for estimating the effects, and even the risk, on algae population to a repeated herbicide stress. This model will be validated by laboratory experiments applying three typical scenarios of pulses exposure. The scenarios differ from each other by the concentration of the different peaks, the exposure duration and, finally, the recovery period between the pulses. The first one corresponds to three pulses of high herbicides concentrations (EC40) and long recovery periods (around 2/3 of the pulse duration). The second scenario corresponds to three pulses of low herbicides concentrations (EC20) and short recovery periods (around 1/3 of the pulse duration). And the last one is a mix of the 2 previous scenarios. These scenarios are tested on the green algae Scenedesmus vacuolatus, which is characteristic of primary producer found in rivers. Two photosynthetic growths are used as test compounds. As endpoint, the growth of algae will be regularly measured during pulses exposure and recovery periods. The model as well as its validation will be discussed.

WEPC6-2
Can time-weighted average concentrations be used to assess the risks of asulam to Myriophyllum spicatum and Elodea canadensis under different time-variable exposure regimes?
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In conclusion, the data suggest that for asulam and macrophytes the TWA approach can be used in the risk assessment.

WEPC6-3
Lemna TKTD-Model for pesticide risk-assessment

SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
The first tier pesticide risk assessment for aquatic macrophytes is to date mainly based on endpoints from standard laboratory tests with a *Lemna* species. To detect even slight effects, the experiments are performed under optimal and static exposure conditions. Both might significantly deviate from the environmental conditions for which the risk assessment is to be performed. Particularly in the case of “non-lethal” endpoints as e.g. reversible growth inhibition it is often an open question in how far effects of an active substance would be different under different conditions with temporally varying exposure and/or slower growth.

We developed a simple growth model simulating the development of dry biomass in dependence of environmental factors as temperature, irradiation and nutrition as well as internal concentration of a growth inhibitor. The internal concentration of the toxicant is calculated from the external exposure with a toxicokinetic (TK) sub-model consisting of a one compartment TK model that includes a mechanistic description of uptake kinetics and partitioning between plant tissue and water.

The functional relationships between growth rate and environmental and internal factors were taken from the published literature. Toxicological parameters for the toxicodynamic model can be derived from standard test results. The rates of uptake into *Lemna*, however, need to be determined in a specifically designed test. For this purpose the uptake of radio-labeled substance into *Lemna* under static exposure as well as the release from loaded plants into uncontaminated water are measured in dependence of time. From the resulting concentration time curves permeability values were derived which make up the essential input parameters for the toxicokinetic sub-model. It is demonstrated that the model can be applied to assess the risk of pesticide applications considering realistic surface-water exposure patterns.

**WEPC-4**

A time-dependent effect model for ecotoxicological effects in *Lemna* species

J. R. Harlan

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The available models for time-dependent effects on aquatic organisms have been recently reviewed and a unified scheme for describing survival has been proposed. However, less recent work has been published on time-dependent effects on gradual endpoints such as growth inhibition of aquatic macrophytes.

The presentation shows a model suitable to describe the time dependence of growth inhibition of *Lemna gilba*. The model allows for the evaluation of the applicability of time-averaged concentration values in risk assessments as well as for a prediction of the effects of time-variable exposure patterns, within certain limits that are derived from the the exposure conditions present in the available experimental data.

**WEPC-5**

TKTD modelling of interactions between toxicants and food - a case study for diquat in the pond snail *Lymnaea stagnalis*

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Toxicokinetic-toxicodynamic (TKTD) models are essential in the interpretation of toxicity tests with time-varying exposure. Furthermore, these models are the only possibility to extrapolate between different exposure scenarios. For effects on survival, the recently published General Unified Threshold model for Survival (GUTS/) can be applied. For effects on growth, such as growth and reproduction, other approaches are needed. At this moment, the only mechanism-based TKTD approaches for these endpoints are derived from the Dynamic Energy Budget (DEB) theory. DEB theory provides a framework that explains how the energy taken up from food is allocated to the major physiological functions at the individual level. Internal concentrations from a TK model can be linked to changes in DEB parameters. Since food can be considered explicitly in a DEB framework, the quality of food and potential interactions with other parameters can be considered. Also, effects on toxicokinetics from the food source on toxicity have been discussed at length in the literature, their impacts are hardly quantitatively assessed. Food limitation often leads to higher apparent sensitivity (e.g., focusing on the EC50), which is not necessarily connected to an increased intrinsic sensitivity (i.e., the relationship between the internal concentration and the value of a DEB parameter).

Recently, it has been found that the pond snail *Lymnaea stagnalis* is food limited in a part of the life-cycle under standardized test conditions, with severe consequences for the interpretation of toxicodynamic toxicity tests. In this study, we use a model based on DEB theory to explain the response of two genetically different populations of the pond snail to pulsed exposure to the herbicide diquat, at four different concentrations. We discuss the interactions between the direct effects of the herbicide on the snails and indirect effects through the food used in the experiments.

**WEPC-6**

Applying the general unified threshold model of survival (GUTS) to describe toxic effects of Triphenylhydroxide on the cyclopoid copepod Mesocyclops leuckarti

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In order to establish good ecological status in all water bodies in the European Union by 2015, the regulators and water managers are currently facing serious problems in identifying the probable causes of surface water impairment and are consequently limited in their ability to predict the impact of their programs of measures in an environment in a quantitative way. In this context, numerous ecological models with different specialization exist and can theoretically be used to support questions concerning watershed management. AQUATOX may simulate the combined environmental fate and effects of anthropogenic compounds or the impacts of nutrient compounds and suspended materials in aquatic ecosystems. AQUATOX is by far the most complete model described in the literature. However, although the model combines aquatic ecosystem, chemical fate and ecotoxicological submodel, it doesn’t model metal fate and effects. Nevertheless, metals originating from urban surface runoff and sewer discharges can be evaluated with tissue residues of metal-tolerant species that correlate with the absence of sensitive species (calibrated monitor approach). The project foresees laboratory investigations on toxicokinetics and lethal and sublethal toxicity on the accumulating biomonitor and selected sensitive species. Validation will be achieved in the field via water monitoring and analysis of residues of bioaccumulative substances besides as macro-invertebrate investigations. Being a planktonic species, *M. leuckarti* is a good representative of aquatic insects and suspended materials in freshwater ecosystems. As aquatic insects are often the dominant macro-invertebrate fauna in freshwater ecosystems, a set of standard laboratory test procedures has been developed to test toxic effects on different life stages of *M. leuckarti* to the organotin compound Triphenylhydroxide (TPT), a non-systemic fungicide, to simulate and interpret its toxic effects. In this study, nauplii, copepods and adults (including males and females, 40 individuals per concentration) were exposed to six different concentrations (12.5, 25, 50, 100, 200 µg/l) of TPT for 96 hours. At nine different time points, the mobility of the copepods was checked. The toxicant exposure concentrations in the test medium were quantified by HPLC. Our results show that a majority of individuals die at exposure concentration higher than 100 µg/l. The LC50 of adult copepods and copepodites were calculated to be between 50 and 60 µg/l. There were no significant differences found between the response of males, females and copepodites to TPT. The generated survival data were analyzed with the “General Unified Threshold Model of Survival” (GUTS), which integrates several toxicokinetic-toxicodynamic models with different assumptions and hypotheses for endpoint survival in one mathematical approach. The model was calibrated with the data set and simulations with several different environmental conditions with mechanistic insight on the concept of individual tolerance versus stochastic death (with and without addition of TPT) were run. The simulation with the best fit to measured survival data was identified. Also, differences between the simulations were interpreted and predictions for the toxicity of TPT on *M. leuckarti* over longer time periods and with miscellaneous exposure patterns were made. The effect-levels (ECx) of TPT were calculated with different assumptions (Logit, Probit, Weibull) of the concentration-response relationship and compared to the outcome of the GUTS-model.

**WEPC-7**

Integration and calibration of a biodynamic toxicity module for metals in the ecological model AQUATOX

S. Massarin, R. Carafa, T. Gallé

CRP Henri Tudor, Esch-sur-alzette, Luxembourg

In order to establish good ecological status in all water bodies in the European Union by 2015, the regulators and water managers are currently facing serious problems in identifying the probable causes of surface water impairment and are consequently limited in their ability to predict the impact of their programs of measures in an environment in a quantitative way. In this context, numerous ecological models with different specialization exist and can theoretically be used to support questions concerning watershed management. AQUATOX may simulate the combined environmental fate and effects of anthropogenic compounds or the impacts of nutrient compounds and suspended materials in aquatic ecosystems. AQUATOX is by far the most complete model described in the literature. However, although the model combines aquatic ecosystem, chemical fate and ecotoxicological submodel, it doesn't model metal fate and effects. Nevertheless, metals originating from urban surface runoff and sewer discharges can be evaluated with tissue residues of metal-tolerant species that correlate with the absence of sensitive species (calibrated monitor approach). The project foresees laboratory investigations on toxicokinetics and lethal and sublethal toxicity on the accumulating biomonitor and selected sensitive species. Validation will be achieved in the field via water monitoring and analysis of residues of bioaccumulative substances besides as macro-invertebrate investigations. Being a planktonic species, *M. leuckarti* is a good representative of aquatic insects and suspended materials in freshwater ecosystems. As aquatic insects are often the dominant macro-invertebrate fauna in freshwater ecosystems, a set of standard laboratory test procedures has been developed to test toxic effects on different life stages of *M. leuckarti* to the organotin compound Triphenylhydroxide (TPT), a non-systemic fungicide, to simulate and interpret its toxic effects. In this study, nauplii, copepods and adults (including males and females, 40 individuals per concentration) were exposed to six different concentrations (12.5, 25, 50, 100, 200 µg/l) of TPT for 96 hours. At nine different time points, the mobility of the copepods was checked. The toxicant exposure concentrations in the test medium were quantified by HPLC. Our results show that a majority of individuals die at exposure concentration higher than 100 µg/l. The LC50 of adult copepods and copepodites were calculated to be between 50 and 60 µg/l. There were no significant differences found between the response of males, females and copepodites to TPT. The generated survival data were analyzed with the “General Unified Threshold Model of Survival” (GUTS), which integrates several toxicokinetic-toxicodynamic models with different assumptions and hypotheses for endpoint survival in one mathematical approach. The model was calibrated with the data set and simulations with several different environmental conditions with mechanistic insight on the concept of individual tolerance versus stochastic death (with and without addition of TPT) were run. The simulation with the best fit to measured survival data was identified. Also, differences between the simulations were interpreted and predictions for the toxicity of TPT on *M. leuckarti* over longer time periods and with miscellaneous exposure patterns were made. The effect-levels (ECx) of TPT were calculated with different assumptions (Logit, Probit, Weibull) of the concentration-response relationship and compared to the outcome of the GUTS-model.

**WEPC-8**

Influence of a variable exposure on Daphnia magna, *Straugi*

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Standard laboratory are generally performed under ‘worst-case’ conditions using most sensitive live stages and maximizing the exposure, e.g. via maintaining constant exposure throughout the entire test duration. Under realistic conditions, however, exposure situations normally differ from those in standard ecotoxicological testing systems since the exposure of water bodies with plant protection products is highly variable due to of different environmental uses, of the use of plant protection products and nature of the receiving water bodies. Therefore variable exposure is a frequent more realistic scenario. Also, tests are done with specific generally most sensitive life-stages; effects on other life stages and thus on the population may differ considerably. In this investigation the effects of a fungicide and the frequently investigated chemical 3,4-dichloroaniline to *Daphnia magna* are examined in different pulsed exposure scenarios with different durations and concentrations. The results of a population test and *Daphnia* reproduction tests with pulsed exposure scenarios (referring to OECD guideline 211) including the investigation of the fitness of the F1 generation are compared.

The results of these pulsed exposure scenarios are in a marked contrast to the standard test using a constant exposure scenario. They demonstrate that the impact of a more realistic variable exposure differs significantly from standard constant exposure studies, particularly for fast acting and rapidly dissipating substances. Additionally, following an initial impact at higher concentrations, a recovery of the populations could be detected after the exposure period.
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